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(21) International Application Number: <b>PCT/US98/15426</b> (22) International Filing Date: 24 July 1998 (24.07.98) (30) Priority Data: 60/055,105 6 August 1997 (06.08.97) <b>US</b> (71) Applicant: <b>CORNELL RESEARCH FOUNDATION, INC.</b> [US/US]; Suite 105, 20 Thornwood Drive, Ithaca, NY 14850 (US). (72) Inventors: <b>BOGDANOVE, Adam, J.</b> ; 210-2 Airport Road, West Lafayette, IN 47906 (US). <b>KIM, Jihyun, Francis</b> ; Apartment E-4, 2250 N. Triphammer Road (US). <b>WEI, Zhong-Min</b> ; 8230 125th Court, Kirkland, WA 98034 (US). <b>BEER, Steven, V.</b> ; 211 Hudson Street, Ithaca, NY 14850 (US). (74) Agents: <b>GOLDMAN, Michael, L. et al.</b> ; Nixon, Hargrave, Devans & Doyle LLP, Clinton Square, P.O. Box 1051, Rochester, NY 14603 (US).			(81) Designated States: <b>AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).</b>  <b>Published</b> <i>With international search report.</i>
(54) Title: <b>HYPERSENSITIVE RESPONSE ELICITOR FROM ERWINIA AMYLOVORA, ITS USE, AND ENCODING GENE</b>			
(57) Abstract <p>The present invention is directed to an isolated protein or polypeptide which elicits a hypersensitive response in plants as well as an isolated DNA molecule which encodes the hypersensitive response eliciting protein or polypeptide. This isolated protein or polypeptide and the isolated DNA molecule can be used to impart disease resistance to plants, to enhance plant growth, and/or to control insects on plants. This can be achieved by applying the hypersensitive response elicitor protein or polypeptide in a non-infectious form to plants or plant seeds under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects on plants or plants grown from the plant seeds. Alternatively, transgenic plants or plant seeds transformed with a DNA molecule encoding a hypersensitive response elicitor protein or polypeptide can be provided and the transgenic plants or plants resulting from the transgenic plant seeds are grown under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects on plants or plants grown from the plant seeds.</p>			

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**HYPERSENSITIVE RESPONSE ELICITOR FROM  
*ERWINIA AMYLOVORA*, ITS USE, AND ENCODING GENE**

This application claims benefit of U.S. Provisional Patent Application  
5 Serial No. 60/055,105, filed August 4, 1997.

**FIELD OF THE INVENTION**

The present invention relates to a hypersensitive response elicitor from  
10 *Erwinia amylovora*, its use, and encoding gene.

**BACKGROUND OF THE INVENTION**

Interactions between bacterial pathogens and their plant hosts generally  
15 fall into two categories: (1) compatible (pathogen-host), leading to intercellular  
bacterial growth, symptom development, and disease development in the host plant;  
and (2) incompatible (pathogen-nonhost), resulting in the hypersensitive response, a  
particular type of incompatible interaction occurring, without progressive disease  
symptoms. During compatible interactions on host plants, bacterial populations  
20 increase dramatically and progressive symptoms occur. During incompatible  
interactions, bacterial populations do not increase, and progressive symptoms do not  
occur.

The hypersensitive response ("HR") is a rapid, localized necrosis that  
is associated with the active defense of plants against many pathogens (Kiraly, Z.,  
25 "Defenses Triggered by the Invader: Hypersensitivity," pages 201-224 in: Plant  
Disease: An Advanced Treatise, Vol. 5, J.G. Horsfall and E.B. Cowling, ed.  
Academic Press New York (1980); Klement, Z., "Hypersensitivity," pages 149-177  
in: Phytopathogenic Prokaryotes, Vol. 2, M.S. Mount and G.H. Lacy, ed. Academic  
Press, New York (1982)). The hypersensitive response elicited by bacteria is readily  
30 observed as a tissue collapse if high concentrations ( $\geq 10^7$  cells/ml) of a limited  
host-range pathogen like *Pseudomonas syringae* or *Erwinia amylovora* are infiltrated  
into the leaves of nonhost plants (necrosis occurs only in isolated plant cells at lower  
levels of inoculum) (Klement, Z., "Rapid Detection of Pathogenicity of  
Phytopathogenic Pseudomonads," Nature 199:299-300; Klement, et al.,

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"Hypersensitive Reaction Induced by Phytopathogenic Bacteria in the Tobacco Leaf,"  
Phytopathology 54:474-477 (1963); Turner, et al., "The Quantitative Relation  
 Between Plant and Bacterial Cells Involved in the Hypersensitive Reaction,"  
Phytopathology 64:885-890 (1974); Klement, Z., "Hypersensitivity," pages 149-177  
 5 in Phytopathogenic Prokaryotes, Vol. 2., M.S. Mount and G.H. Lacy, ed. Academic  
 Press, New York (1982)). The capacities to elicit the hypersensitive response in a  
 nonhost and be pathogenic in a host appear linked. As noted by Klement, Z.,  
 "Hypersensitivity," pages 149-177 in Phytopathogenic Prokaryotes, Vol. 2., M.S.  
 Mount and G.H. Lacy, ed. Academic Press, New York, these pathogens also cause  
 10 physiologically similar, albeit delayed, necroses in their interactions with compatible  
 hosts. Furthermore, the ability to produce the hypersensitive response or pathogenesis  
 is dependent on a common set of genes, denoted *hrp* (Lindgren, P.B., et al., "Gene  
 Cluster of *Pseudomonas syringae* pv. 'phaseolicola' Controls Pathogenicity of Bean  
 Plants and Hypersensitivity on Nonhost Plants," J. Bacteriol. 168:512-22 (1986);  
 15 Willis, D.K., et al., "*hrp* Genes of Phytopathogenic Bacteria," Mol. Plant-Microbe  
Interact. 4:132-138 (1991)). Consequently, the hypersensitive response may hold  
 clues to both the nature of plant defense and the basis for bacterial pathogenicity.

The *hrp* genes are widespread in gram-negative plant pathogens, where  
 they are clustered, conserved, and in some cases interchangeable (Willis, D.K., et al.,  
 20 "*hrp* Genes of Phytopathogenic Bacteria," Mol. Plant-Microbe Interact. 4:132-138  
 (1991); Bonas, U., "*hrp* Genes of Phytopathogenic Bacteria," pages 79-98 in: Current  
Topics in Microbiology and Immunology: Bacterial Pathogenesis of Plants and  
Animals - Molecular and Cellular Mechanisms, J.L. Dangel, ed. Springer-Verlag,  
 Berlin (1994)). Several *hrp* genes encode components of a protein secretion pathway  
 25 similar to one used by *Yersinia*, *Shigella*, and *Salmonella* spp. to secrete proteins  
 essential in animal diseases (Van Gijsegem, et al., "Evolutionary Conservation of  
 Pathogenicity Determinants Among Plant and Animal Pathogenic Bacteria," Trends  
Microbiol. 1:175-180 (1993)). In *E. amylovora*, *P. syringae*, and *P. solanacearum*,  
*hrp* genes have been shown to control the production and secretion of glycine-rich,  
 30 protein elicitors of the hypersensitive response (He, S.Y., et al. "Pseudomonas  
*Syringae* pv. *Syringae* Harpin<sub>PSS</sub>: a Protein that is Secreted via the Hrp Pathway and  
 Elicits the Hypersensitive Response in Plants," Cell 73:1255-1266 (1993). Wei, Z.-H.,

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et al., "HrpI of *Erwinia amylovora* Functions in Secretion of Harpin and is a Member of a New Protein Family," J. Bacteriol. 175:7958-7967 (1993); Arlat, M. et al. "PopA1, a Protein Which Induces a Hypersensitive-like Response on Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-553 (1994)).

The first of these proteins was discovered in *E. amylovora* Ea321, a bacterium that causes fire blight of rosaceous plants, and was designated harpin (Wei, Z.-M., et al, "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992)). Mutations in the encoding *hrpN* gene revealed that the hypersensitive response elicitor is required for *E. amylovora* to elicit a hypersensitive response in nonhost tobacco leaves and incite disease symptoms in highly susceptible pear fruit. The *P. solanacearum* GMI1000 PopA1 protein has similar physical properties and also elicits the hypersensitive response in leaves of tobacco, which is not a host of that strain (Arlat, et al. "PopA1, a Protein Which Induces a Hypersensitive-like Response on Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-53 (1994)). However, *P. solanacearum popA* mutants still elicit the hypersensitive response in tobacco and incite disease in tomato. Thus, the role of these glycine-rich hypersensitive response elicitors can vary widely among gram-negative plant pathogens.

Other plant pathogenic hypersensitive response elicitors have been isolated and their encoding genes have been cloned and sequenced. These include: *Erwinia chrysanthemi* (Bauer, et. al., "*Erwinia chrysanthemi* Harpin<sub>Ech</sub>: Soft-Rot Pathogenesis," MPMI 8(4): 484-91 (1995)); *Erwinia carotovora* (Cui, et. al., "The RsmA<sup>-</sup> Mutants of *Erwinia carotovora* subsp. *carotovora* Strain Ecc71 Overexpress *hrpN*<sub>Ecc</sub> and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves," MPMI 9(7): 565-73 (1966)); *Erwinia stewartii* (Ahmad, et. al., "Harpin is not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," 8th Int'l. Cong. Molec. Plant-Microb. Inter. July 14-19, 1996 and Ahmad, et. al., "Harpin is not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," Ann. Mtg. Am. Phytopath. Soc. July 27-31, 1996); and *Pseudomonas syringae* pv. *syringae* (WO 94/26782 to Cornell Research Foundation, Inc.).

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The present invention is a further advance in the effort to identify, clone, and sequence hypersensitive response elicitor proteins or polypeptides from plant pathogens.

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## SUMMARY OF THE INVENTION

The present invention is directed to an isolated protein or polypeptide which elicits a hypersensitive response in plants as well as an isolated DNA molecule which encodes the hypersensitive response eliciting protein or polypeptide.

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The hypersensitive response eliciting protein or polypeptide can be used to impart disease resistance to plants, to enhance plant growth, and/or to control insects. This involves applying the hypersensitive response elicitor protein or polypeptide in a non-infectious form to plants or plant seeds under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects on plants or plants grown from the plant seeds.

15

As an alternative to applying the hypersensitive response elicitor protein or polypeptide to plants or plant seeds in order to impart disease resistance, to enhance plant growth, and/or to control insects on plants, transgenic plants or plant seeds can be utilized. When utilizing transgenic plants, this involves providing a transgenic plant transformed with a DNA molecule encoding a hypersensitive response elicitor protein or polypeptide and growing the plant under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects in the plants or plants grown from the plant seeds. Alternatively, a transgenic plant seed transformed with the DNA molecule encoding a hypersensitive response elicitor protein or polypeptide can be provided and planted in soil. A plant is then propagated under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects on plants or plants grown from the plant seeds.

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## BRIEF DESCRIPTION OF THE DRAWINGS

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Figures 1A-D show mutagenesis, complementation and heterologous expression constructs, and homology with and complementation of mutants by the *avrE* locus of *P. syringae* for the *dspE* operon of *E. amylovora*. Dashed boxes are

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uncharacterized ORFs; a filled triangle indicates a *hrp* (i.e. hypersensitive response elicitor encoding gene); box is a regulatory sequence that precedes many *hrp* genes; and an open triangle indicates another promoter. Thick lines delineate the DNA for which sequence was accessioned. Figure 1A shows the *dsp/hrp* gene cluster of

5 *E. amylovora* in pCPP430. Operon names and types of proteins encoded are indicated at the top. B, *Bam*HI; E, *Eco*RI; H, *Hind*III. Half-arrows indicate internal promoters without similarity to the *hrp* box consensus. Figure 1B shows the region downstream of *hrpN* containing the *dspE* operon. Circles mark deletion mutations and representative transposon insertions: black, non-pathogenic and HR<sup>+</sup> (i.e.

10 hypersensitive response eliciting) or HR reduced (*dsp*); gray, reduced virulence and HR; white, wild-type. T104 lies within the area marked by the dashed double arrow. K, *Tn5*miniKm; P, *Tn5*phoA; T, *Tn10*tet<sup>r</sup>; Δ, deletion mutation. The gray box is A/T-rich DNA. Figure 1C shows the clones and subclones of the *dspE* operon. Plasmid designations are indicated at the left, and vector-borne promoters at the right.

15 Restriction sites used for subcloning not shown above are shown in parentheses. A “+” aligned with a circle representing a mutation in B indicates that the subclone complements that mutation. Figure 1D shows the *avrE* locus (transcription units III and IV) of *P. syringae* pv. tomato DC3000 in pCPP2357. Percent amino acid identity of the predicted proteins AvrE and AvrF to DspE and DspF, respectively, are

20 indicated. Black rectangles are transcriptional terminators (inverted repeats). Complementation of mutations shown in Figure 1B are depicted as in Figure 1C, above.

Figure 2 shows the expression of the full-length and the N-terminal half of DspE in recombinant *E. coli* DH5α. Lysates of cells carrying either

25 pCPP1259, containing the entire *dspE* operon (lane A); pCPP50, the cloning vector (lane B); or pCPP1244, containing only the 5' half of the *dspE* gene (lane C), were subjected to SDS-PAGE followed by Coomassie staining. Bands corresponding to DspE (lane A) and the N-terminal half of DspE (lane C) are marked by arrows. Migration of molecular weight markers is indicated on the left.

30 Figures 3A-D show the role of *dspe* in pathogenicity and HR elicitation. Figure 3A shows immature pear fruit 4 days after inoculation with (left to right) strains Ea321, Ea321*dspE*Δ1554, or Ea321*dspE*Δ1554 harboring the 5' half of

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*dspE* on pCPP1237. Figure 3B shows Norchief soybean leaf 24 hr after infiltration with (1) Ea321, (2) Ea321*dspE*Δ1554, (3) Ea321*hrpN::Tn5* (Wei, et al., Science, 257:85-88 (1992), which is hereby incorporated by reference), and (4) Ea321*hrpL::Tn5* (Wei, et al., J. Bacteriol., 177:6201-10 (1995), which is hereby incorporated by reference). Figure 3C shows a tobacco leaf 48 hr after infiltration with parallel dilution series of suspensions of strains (left) Ea321 and (right) Ea321*dspE*Δ1554. The concentrations infiltrated (top to bottom) are  $1 \times 10^{10}$ ,  $1 \times 10^9$ ,  $5 \times 10^8$ , and  $5 \times 10^7$  cfu/ml. Figure 3D is like Figure 3C except the more virulent strain Ea273 and corresponding mutant Ea273*dspE*Δ1554 were used, and concentrations ranged from  $5 \times 10^9$  to  $5 \times 10^5$  cfu/ml in log increments.

Figure 4 shows the expression of a promoterless GUS construct fused to *dspE* in *E. amylovora* Ea273. Ea273 and Ea273*dspE::uidA* (a merodiploid containing both a wild-type *dspE* and a truncated *dspE* fused to the *uidA* gene; black bars) were grown in LB or Hrp MM, or inoculated to immature pear fruit. Ea273*dspE::uidA**hrpL::Tn5* (dark gray bar) and Ea273*hrcV::Tn5uidA* (light gray bar) were also grown in *hrp* MM. Values shown represent means of triplicate samples normalized for bacterial cell concentration. Standard deviations are represented by lines extending from each bar. The mean values for three samples of Ea273 in each assay were subtracted from, and standard deviations added to, the corresponding values obtained for the other strains.

Figures 5A-C show the transgeneric avirulence function of the *dspE* operon and complementation of a *dspE* mutant with the *avrE* locus. Norchief soybean leaves were either (See Figure 5A) infiltrated with  $1 \times 10^8$  cfu/ml suspensions of (left) *P. syringae* pv. *glycinea* race 4 carrying pCPP1250 (containing the *dspE* operon) or (right) pML 122 (the cloning vector) and photographed after 24 hr at room temperature or (See Figure 5B) infiltrated with  $8 \times 10^5$  cfu/ml suspensions of the same strains and photographed after seven days at 22° C and high relative humidity. Tissue collapse is apparent on both leaves where the strain carrying pCPP1250 was infiltrated. On the leaf incubated for seven days, chlorosis extending beyond the infiltrated area, typical of disease, is apparent on the half infiltrated with the strain carrying the vector only. The dark section on the side of the leaf infiltrated with the strain carrying pCPP1250 is a shadow caused by a buckle in the leaf. Figure 5C



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shows pear halves inoculated with (left to right) Ea321, Ea321*dspE*Δ1521(pCPP2357, containing the *avrE* locus), or Ea321*dspE*Δ1521(pCPP2357*avrE::TnSuidA*) and photographed after seven days. Although symptoms are greatly reduced relative to wild type, necrosis is apparent around and drops of ooze can be seen within the well of the fruit inoculated with the *dspE* strain carrying the intact *avrE* locus. The fruit inoculated with the *dspE* strain carrying a disrupted clone of *avrE* is symptomless.

### DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to an isolated DNA molecule having a nucleotide sequence of SEQ. ID. No. 1 as follows:

	ATGGAATTAA AATCACTGGG AACTGAACAC AAGGCGGCAG TACACACAGC GGCGCACAAAC	60
15	CCTGTGGGGC ATGGTGTGTC CTTACAGCAG GGCAGCAGCA GCAGCAGCCC GCAAAATGCC	120
	GCTGCATCAT TGGCGGCAGA AGGCAAAAAT CGTGGGAAAA TGCCGAGAAT TCACCAGCCA	180
20	TCTACTGCGG CTGATGGTAT CAGCGCTGCT CACCAGCAAA AGAAATCCTT CAGTCTCAGG	240
	GGCTGTTTGG GGACGAAAAA ATTTTCCAGA TCGGCACCGC AGGGCCAGCC AGGTACCACC	300
	CACAGCAAAG GGGCAACATT GCGCGATCTG CTGGCGCGGG ACGACGGCGA AACGCAGCAT	360
25	GAGGCGGCCG CGCCAGATGC GGCGCGTTTG ACCCGTTCCG GCGGCGTCAA ACGCCGCAAT	420
	ATGGACGACA TGGCCGGGCG GCCAATGGTG AAAGGTGGCA GCGGCGAAGA TAAGGTACCA	480
30	ACGCAGCAAA AACGGCATCA GCTGAACAAT TTTGGCCAGA TGCGCCAAAC GATGTTGAGC	540
	AAAATGGCTC ACCCGGCTTC AGCCAACGCC GCGGATCGCC TGCAGCATTC ACCGCCGCAC	600
	ATCCCGGGTA GCCACCAGCA AATCAAGGAA GAACCGGTTG GCTCCACCAG CAAGGCAACA	660
35	ACGGCCACG CAGACAGAGT GGAAATCGCT CAGGAAGATG ACGACAGCGA ATTCCAGCAA	720
	CTGCATCAAC AGCGGCTGGC GCGCGAACGG GAAAATCCAC CGCAGCCGCC CAAACTCGGC	780
40	GTTGCCACAC CGATTAGCGC CAGGTTTCAG CCCAACTGA CTGCGGTTGC GGAAAGCGTC	840
	CTTGAGGGGA CAGATACCAC GCAGTCACCC CTTAAGCCGC AATCAATGCT GAAAGGAAGT	900
	GGAGCCGGGG TAACGCCGCT GCGGTAACG CTGGATAAAG GCAAGTTGCA GCTGGCACCG	960
45	GATAATCCAC CCGCGCTCAA TACGTTGTG AAGCAGACAT TGGGTAAAGA CACCCAGCAC	1020
	TATCTGGCGC ACCATGCCAG CAGCGACGGT AGCCAGCATC TGCTGCTGGA CAACAAAGGC	1080
50	CACCTGTTTG ATATCAAAAG CACCGCCACC AGCTATAGCG TGCTGCACAA CAGCCACCCC	1140
	GGTGAGATAA AGGGCAAGCT GGCGCAGGCG GGTACTGGCT CCGTCAGCGT AGACGGTAAA	1200

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	AGCGGCAAGA TCTCGCTGGG GAGCGGTACG CAAAGTCACA ACAAACAAT GCTAAGCCAA	1260
	CCGGGGGAAG CGCACCGTTC CTTATTAACC GGCATTTGGC AGCATCCTGC TGGCGCAGCG	1320
5	CGGCCGCAGG GCGAGTCAAT CCGCCTGCAT GACGACAAAA TTCATATCCT GCATCCGGAG	1380
	CTGGGCGTAT GGCAATCTGC GGATAAAGAT ACCCACAGCC AGCTGTCTCG CCAGGCAGAC	1440
10	GGTAAGCTCT ATGCGCTGAA AGACAACCGT ACCCTGCAAA ACCTCTCCGA TAATAAATCC	1500
	TCAGAAAAGC TGGTCGATAA AATCAAATCG TATTCGGTTG ATCAGCGGGG GCAGGTGGCG	1560
	ATCCTGACGG ATACTCCCGG CCGCCATAAG ATGAGTATTA TGCCCTCGCT GGATGCTTCC	1620
15	CCGGAGAGCC ATATTTCCCT CAGCCTGCAT TTTGCCGATG CCCACCAGGG GTTATTGCAC	1680
	GGGAAGTCGG AGCTTGAGGC ACAATCTGTC GCGATCAGCC ATGGGCGACT GGTGTGGCC	1740
20	GATAGCGAAG GCAAGCTGTT TAGCGCCGCC ATTCCGAAGC AAGGGGATGG AAACGAACTG	1800
	AAAATGAAAG CCATGCCTCA GCATGCGCTC GATGAACATT TTGGTCATGA CCACCAGATT	1860
	TCTGGATTTT TCCATGACGA CCACGGCCAG CTTAATGCGC TGGTGAAAAA TAACTTCAGG	1920
25	CAGCAGCATG CCTGCCCGTT GGGTAACGAT CATCAGTTTC ACCCCGGCTG GAACCTGACT	1980
	GATGCGCTGG TTATCGACAA TCAGCTGGGG CTGCATCATA CCAATCCTGA ACCGCATGAG	2040
30	ATTCTTGATA TGGGGCATTT AGGCAGCCTG GCGTTACAGG AGGGCAAGCT TCACTATTTT	2100
	GACCAGCTGA CCAAAGGGTG GACTGGCGCG GAGTCAGATT GTAAGCAGCT GAAAAAGGC	2160
	CTGGATGGAG CAGCTTATCT ACTGAAAGAC GGTGAAGTGA AACGCCTGAA TATTAATCAG	2220
35	AGCACCTCCT CTATCAAGCA CGGAACGGAA AACGTTTTTT CGCTGCCGCA TGTGCGCAAT	2280
	AAACCGGAGC CGGGAGATGC CCTGCAAGGG CTGAATAAAG ACGATAAGGC CCAGGCCATG	2340
40	GCGGTGATTG GGGTAAATAA ATACCTGGCG CTGACGGAAG AAGGGGACAT TCGCTCCTTC	2400
	CAGATAAAAC CCGGCACCCA GCAGTTGGAG CGGCCGGCAC AAATCTCAG CCGCGAAGGT	2460
	ATCAGCGGCG AACTGAAAGA CATTATGTC GACCACAAGC AGAACCTGTA TGCCTTGACC	2520
45	CACGAGGGAG AGGTGTTTCA TCAGCCGCGT GAAGCCTGGC AGAATGGTGC CGAAAGCAGC	2580
	AGCTGGCACA AACTGGCGTT GCCACAGAGT GAAAGTAAGC TAAAAAGTCT GGACATGAGC	2640
50	CATGAGCACA AACCGATTGC CACCTTTGAA GACGGTAGCC AGCATCAGCT GAAGGCTGGC	2700
	GGCTGGCAGC CCTATGCGGC ACCTGAACGC GGGCCGCTGG CCGTGGGTAC CAGCGGTTCA	2760
	CAAACCGTCT TTAACCGACT AATGCAGGGG GTGAAAGGCA AGGTGATCCC AGGCAGCGGG	2820
55	TTGACGGTTA AGCTCTCGGC TCAGACGGGG GGAATGACCG GCGCCGAAGG GCGCAAGGTC	2880
	AGCAGTAAAT TTTCCGAAAG GATCCGCGCC TATGCGTTCA ACCCAACAAT GTCCACGCCG	2940
60	CGACCGATTA AAAATGCTGC TTATGCCACA CAGCACGGCT GGCAGGGGCG TGAGGGGTTG	3000
	AAGCCGTTGT ACGAGATGCA GGGAGCGCTG ATTAACAAC TGGATGCGCA TAACGTTCTG	3060

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	CATAACGCGC CACAGCCAGA TTTGCAGAGC AAACCTGGAAA CTCTGGATTT AGGCGAACAT	3120
	GGCGCAGAAT TGCTTAACGA CATGAAGCGC TTCCGCGACG AACTGGAGCA GAGTGCAACC	3180
5	CGTTCGGTGA CCGTTTTAGG TCAACATCAG GGAGTGCTAA AAAGCAACGG TGAAATCAAT	3240
	AGCGAATTTA AGCCATCGCC CGGCAAGGCG TTGGTCCAGA GCTTTAACGT CAATCGCTCT	3300
10	GGTCAGGATC TAAGCAAGTC ACTGCAACAG GCAGTACATG CCACGCCGCC ATCCGCAGAG	3360
	AGTAAACTGC AATCCATGCT GGGGCACTTT GTCAGTGCCG GGGTGGATAT GAGTCATCAG	3420
	AAGGGCGAGA TCCCGCTGGG CCGCCAGCGC GATCCGAATG ATAAAACCGC ACTGACCAAA	3480
15	TCGCGTTTAA TTTTAGATAC CGTGACCATC GGTGAACTGC ATGAACTGGC CGATAAGGCG	3540
	AAACTGGTAT CTGACCATAA ACCCGATGCC GATCAGATAA AACAGCTGCG CCAGCAGTTC	3600
20	GATACGCTGC GTGAAAAGCG GTATGAGAGC AATCCGGTGA AGCATTACAC CGATATGGGC	3660
	TTCACCCATA ATAAGGCGCT GGAAGCAAAC TATGATGCGG TCAAAGCCTT TATCAATGCC	3720
	TTTAAGAAAG AGCACCACGG CGTCAATCTG ACCACGCGTA CCGTACTGGA ATCACAGGGC	3780
25	AGTGCGGAGC TGGCGAAGAA GCTCAAGAAT ACGCTGTTGT CCCTGGACAG TGGTGAAAGT	3840
	ATGAGCTTCA GCCGGTCATA TGGCGGGGGC GTCAGCACTG TCTTTGTGCC TACCCTTAGC	3900
30	AAGAAGGTGC CAGTTCGGT GATCCCGGA GCCGGCATCA CGCTGGATCG CGCCTATAAC	3960
	CTGAGCTTCA GTCGTACCAG CGGCGGATTG AACGTCAGTT TTGGCCGCGA CGGCGGGGTG	4020
	AGTGGAACA TCATGGTCG TACCGGCCAT GATGTGATGC CCTATATGAC CGGTAAGAAA	4080
35	ACCACTGCAG GTAACGCCAG TGAAGGTTG AGCGCAAAAC ATAAAATCAG CCCGACTTG	4140
	CGTATCGGCG CTGCTGTGAG TGGCACCTG CAAGGAACGC TACAAAACAG CCTGAAGTTT	4200
40	AAGCTGACAG AGGATGAGCT GCCTGGCTTT ATCCATGGCT TGACGCATGG CACGTTGACC	4260
	CCGGCAGAAC TGTTGCAAAA GGGGATCGAA CATCAGATGA AGCAGGGCAG CAAACTGACG	4320
	TTTAGCGTCG ATACCTCGGC AAATCTGGAT CTGCGTGCCG GTATCAATCT GAACGAAGAC	4380
45	GGCAGTAAAC CAAATGGTGT CACTGCCCGT GTTCTGCGG GGCTAAGTGC ATCGGCAAC	4440
	CTGGCCGCCG GCTCGCGTGA ACGCAGCACC ACCTCTGGCC AGTTTGGCAG CACGACTTCG	4500
50	GCCAGCAATA ACCGCCCAAC CTTCTCAAC GGGGTCGGCG CGGGTGCTAA CCTGACGGCT	4560
	GCTTTAGGGG TTGCCCATTC ATCTACGCAT GAAGGAAAC CGGTGGGAT CTTCCCGCA	4620
	TTTACCTCGA CCAATGTTTC GGCAGCGCTG GCGCTGGATA ACCGTACCTC ACAGAGTATC	4680
55	AGCCTGGAAT TGAAGCGCGG GGAGCCGGTG ACCAGCAACG ATATCAGCGA GTTGACCTCC	4740
	ACGCTGGGAA AACACTTTAA GGATAGCGCC ACAACGAAGA TGCTTGCCGC TCTCAAAGAG	4800
60	TTAGATGACG CTAAGCCCGC TGAACAACTG CATATTTTAC AGCAGCATTT CAGTGCAAAA	4860
	GATGTCGTCG GTGATGAACG CTACGAGGCG GTGCGCAACC TGAAAAAACT GGTGATACGT	4920

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CAACAGGCTG CGGACAGCCA CAGCATGGAA TTAGGATCTG CCACTCACAG CACGACCTAC 4980  
 AATAATCTGT CGAGAATAAA TAATGACGGC ATTGTCGAGC TGCTACACAA ACATTTTCGAT 5040  
 5 GCGGCATTAC CAGCAAGCAG TGCCAAACGT CTTGGTGAAA TGATGAATAA CGATCCGGCA 5100  
 CTGAAAGATA TTATTAAGCA GCTGCAAAGT ACGCCGTTCA GCAGCGCCAG CGTGTCTGATG 5160  
 GAGCTGAAAG ATGGTCTGCG TGAGCAGACG GAAAAAGCAA TACTGGACGG TAAGGTCGGT 5220  
 10 CGTGAAGAAG TGGGAGTACT TTTCCAGGAT CGTAACAACT TCGGTGTTAA ATCGGTCAGC 5280  
 GTCAGTCAGT CCGTCAGCAA AAGCGAAGGC TTCAATACCC CAGCGCTGTT ACTGGGGACG 5340  
 15 AGCAACAGCG CTGCTATGAG CATGGAGCGC AACATCGGAA CCATTAATTT TAAATACGGC 5400  
 CAGGATCAGA ACACCCACG GCGATTTACC CTGGAGGGTG GAATAGCTCA GGCTAATCCG 5460  
 CAGGTCGCAT CTGCGCTTAC TGATTTGAAG AAGGAAGGGC TGGAAATGAA GAGCTAA 5517  
 20

This DNA molecule is known as the dspE gene. This isolated DNA molecule of the present invention encodes a protein or polypeptide which elicits a plant pathogen's hypersensitive response having an amino acid sequence of SEQ. ID. No. 2 as follows:

25 Met Glu Leu Lys Ser Leu Gly Thr Glu His Lys Ala Ala Val His Thr  
 1 5 10 15  
 30 Ala Ala His Asn Pro Val Gly His Gly Val Ala Leu Gln Gln Gly Ser  
 20 25 30  
 Ser Ser Ser Ser Pro Gln Asn Ala Ala Ala Ser Leu Ala Ala Glu Gly  
 35 35 40 45  
 35 Lys Asn Arg Gly Lys Met Pro Arg Ile His Gln Pro Ser Thr Ala Ala  
 50 55 60  
 40 Asp Gly Ile Ser Ala Ala His Gln Gln Lys Lys Ser Phe Ser Leu Arg  
 65 70 75 80  
 40 Gly Cys Leu Gly Thr Lys Lys Phe Ser Arg Ser Ala Pro Gln Gly Gln  
 85 90 95  
 45 Pro Gly Thr Thr His Ser Lys Gly Ala Thr Leu Arg Asp Leu Leu Ala  
 100 105 110  
 Arg Asp Asp Gly Glu Thr Gln His Glu Ala Ala Ala Pro Asp Ala Ala  
 115 120 125  
 50 Arg Leu Thr Arg Ser Gly Gly Val Lys Arg Arg Asn Met Asp Asp Met  
 130 135 140  
 55 Ala Gly Arg Pro Met Val Lys Gly Gly Ser Gly Glu Asp Lys Val Pro  
 145 150 155 160  
 Thr Gln Gln Lys Arg His Gln Leu Asn Asn Phe Gly Gln Met Arg Gln  
 165 170 175

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	Thr Met Leu Ser Lys Met Ala His Pro Ala Ser Ala Asn Ala Gly Asp	
	180	185 190
5	Arg Leu Gln His Ser Pro Pro His Ile Pro Gly Ser His His Glu Ile	
	195	200 205
	Lys Glu Glu Pro Val Gly Ser Thr Ser Lys Ala Thr Thr Ala His Ala	
	210	215 220
10	Asp Arg Val Glu Ile Ala Gln Glu Asp Asp Asp Ser Glu Phe Gln Gln	
	225	230 235 240
	Leu His Gln Gln Arg Leu Ala Arg Glu Arg Glu Asn Pro Pro Gln Pro	
		245 250 255
15	Pro Lys Leu Gly Val Ala Thr Pro Ile Ser Ala Arg Phe Gln Pro Lys	
		260 265 270
20	Leu Thr Ala Val Ala Glu Ser Val Leu Glu Gly Thr Asp Thr Thr Gln	
		275 280 285
	Ser Pro Leu Lys Pro Gln Ser Met Leu Lys Gly Ser Gly Ala Gly Val	
		290 295 300
25	Thr Pro Leu Ala Val Thr Leu Asp Lys Gly Lys Leu Gln Leu Ala Pro	
		305 310 315 320
	Asp Asn Pro Pro Ala Leu Asn Thr Leu Leu Lys Gln Thr Leu Gly Lys	
		325 330 335
30	Asp Thr Gln His Tyr Leu Ala His His Ala Ser Ser Asp Gly Ser Gln	
		340 345 350
35	His Leu Leu Leu Asp Asn Lys Gly His Leu Phe Asp Ile Lys Ser Thr	
		355 360 365
	Ala Thr Ser Tyr Ser Val Leu His Asn Ser His Pro Gly Glu Ile Lys	
		370 375 380
40	Gly Lys Leu Ala Gln Ala Gly Thr Gly Ser Val Ser Val Asp Gly Lys	
		385 390 395 400
	Ser Gly Lys Ile Ser Leu Gly Ser Gly Thr Gln Ser His Asn Lys Thr	
		405 410 415
45	Met Leu Ser Gln Pro Gly Glu Ala His Arg Ser Leu Leu Thr Gly Ile	
		420 425 430
50	Trp Gln His Pro Ala Gly Ala Ala Arg Pro Gln Gly Glu Ser Ile Arg	
		435 440 445
	Leu His Asp Asp Lys Ile His Ile Leu His Pro Glu Leu Gly Val Trp	
		450 455 460
55	Gln Ser Ala Asp Lys Asp Thr His Ser Gln Leu Ser Arg Gln Ala Asp	
		465 470 475 480
	Gly Lys Leu Tyr Ala Leu Lys Asp Asn Arg Thr Leu Gln Asn Leu Ser	
		485 490 495
60	Asp Asn Lys Ser Ser Glu Lys Leu Val Asp Lys Ile Lys Ser Tyr Ser	
		500 505 510

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	Val Asp Gln Arg Gly Gln Val Ala Ile Leu Thr Asp Thr Pro Gly Arg
	515 520 525
5	His Lys Met Ser Ile Met Pro Ser Leu Asp Ala Ser Pro Glu Ser His
	530 535 540
	Ile Ser Leu Ser Leu His Phe Ala Asp Ala His Gln Gly Leu Leu His
10	545 550 555 560
	Gly Lys Ser Glu Leu Glu Ala Gln Ser Val Ala Ile Ser His Gly Arg
	565 570 575
15	Leu Val Val Ala Asp Ser Glu Gly Lys Leu Phe Ser Ala Ala Ile Pro
	580 585 590
	Lys Gln Gly Asp Gly Asn Glu Leu Lys Met Lys Ala Met Pro Gln His
	595 600 605
20	Ala Leu Asp Glu His Phe Gly His Asp His Gln Ile Ser Gly Phe Phe
	610 615 620
	His Asp Asp His Gly Gln Leu Asn Ala Leu Val Lys Asn Asn Phe Arg
25	625 630 635 640
	Gln Gln His Ala Cys Pro Leu Gly Asn Asp His Gln Phe His Pro Gly
	645 650 655
30	Trp Asn Leu Thr Asp Ala Leu Val Ile Asp Asn Gln Leu Gly Leu His
	660 665 670
	His Thr Asn Pro Glu Pro His Glu Ile Leu Asp Met Gly His Leu Gly
	675 680 685
35	Ser Leu Ala Leu Gln Glu Gly Lys Leu His Tyr Phe Asp Gln Leu Thr
	690 695 700
	Lys Gly Trp Thr Gly Ala Glu Ser Asp Cys Lys Gln Leu Lys Lys Gly
40	705 710 715 720
	Leu Asp Gly Ala Ala Tyr Leu Leu Lys Asp Gly Glu Val Lys Arg Leu
	725 730 735
45	Asn Ile Asn Gln Ser Thr Ser Ser Ile Lys His Gly Thr Glu Asn Val
	740 745 750
	Phe Ser Leu Pro His Val Arg Asn Lys Pro Glu Pro Gly Asp Ala Leu
	755 760 765
50	Gln Gly Leu Asn Lys Asp Asp Lys Ala Gln Ala Met Ala Val Ile Gly
	770 775 780
	Val Asn Lys Tyr Leu Ala Leu Thr Glu Lys Gly Asp Ile Arg Ser Phe
55	785 790 795 800
	Gln Ile Lys Pro Gly Thr Gln Gln Leu Glu Arg Pro Ala Gln Thr Leu
	805 810 815
60	Ser Arg Glu Gly Ile Ser Gly Glu Leu Lys Asp Ile His Val Asp His
	820 825 830

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Lys Gln Asn Leu Tyr Ala Leu Thr His Glu Gly Glu Val Phe His Gln  
 835 840 845  
 5 Pro Arg Glu Ala Trp Gln Asn Gly Ala Glu Ser Ser Ser Trp His Lys  
 850 855 860  
 Leu Ala Leu Pro Gln Ser Glu Ser Lys Leu Lys Ser Leu Asp Met Ser  
 865 870 875 880  
 10 His Glu His Lys Pro Ile Ala Thr Phe Glu Asp Gly Ser Gln His Gln  
 885 890 895  
 Leu Lys Ala Gly Gly Trp His Ala Tyr Ala Ala Pro Glu Arg Gly Pro  
 900 905 910  
 15 Leu Ala Val Gly Thr Ser Gly Ser Gln Thr Val Phe Asn Arg Leu Met  
 915 920 925  
 Gln Gly Val Lys Gly Lys Val Ile Pro Gly Ser Gly Leu Thr Val Lys  
 930 935 940  
 20 Leu Ser Ala Gln Thr Gly Gly Met Thr Gly Ala Glu Gly Arg Lys Val  
 945 950 955 960  
 25 Ser Ser Lys Phe Ser Glu Arg Ile Arg Ala Tyr Ala Phe Asn Pro Thr  
 965 970 975  
 Met Ser Thr Pro Arg Pro Ile Lys Asn Ala Ala Tyr Ala Thr Gln His  
 980 985 990  
 30 Gly Trp Gln Gly Arg Glu Gly Leu Lys Pro Leu Tyr Glu Met Gln Gly  
 995 1000 1005  
 Ala Leu Ile Lys Gln Leu Asp Ala His Asn Val Arg His Asn Ala Pro  
 1010 1015 1020  
 35 Gln Pro Asp Leu Gln Ser Lys Leu Glu Thr Leu Asp Leu Gly Glu His  
 1025 1030 1035 1040  
 40 Gly Ala Glu Leu Leu Asn Asp Met Lys Arg Phe Arg Asp Glu Leu Glu  
 1045 1050 1055  
 Gln Ser Ala Thr Arg Ser Val Thr Val Leu Gly Gln His Gln Gly Val  
 1060 1065 1070  
 45 Leu Lys Ser Asn Gly Glu Ile Asn Ser Glu Phe Lys Pro Ser Pro Gly  
 1075 1080 1085  
 Lys Ala Leu Val Gln Ser Phe Asn Val Asn Arg Ser Gly Gln Asp Leu  
 1090 1095 1100  
 50 Ser Lys Ser Leu Gln Gln Ala Val His Ala Thr Pro Pro Ser Ala Glu  
 1105 1110 1115 1120  
 55 Ser Lys Leu Gln Ser Met Leu Gly His Phe Val Ser Ala Gly Val Asp  
 1125 1130 1135  
 Met Ser His Gln Lys Gly Glu Ile Pro Leu Gly Arg Gln Arg Asp Pro  
 1140 1145 1150  
 60 Asn Asp Lys Thr Ala Leu Thr Lys Ser Arg Leu Ile Leu Asp Thr Val  
 1155 1160 1165

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Thr Ile Gly Glu Leu His Glu Leu Ala Asp Lys Ala Lys Leu Val Ser  
 1170 1175 1180

5 Asp His Lys Pro Asp Ala Asp Gln Ile Lys Gln Leu Arg Gln Gln Phe  
 1185 1190 1195 1200

Asp Thr Leu Arg Glu Lys Arg Tyr Glu Ser Asn Pro Val Lys His Tyr  
 1205 1210 1215

10 Thr Asp Met Gly Phe Thr His Asn Lys Ala Leu Glu Ala Asn Tyr Asp  
 1220 1225 1230

Ala Val Lys Ala Phe Ile Asn Ala Phe Lys Lys Glu His His Gly Val  
 1235 1240 1245

Asn Leu Thr Thr Arg Thr Val Leu Glu Ser Gln Gly Ser Ala Glu Leu  
 1250 1255 1260

20 Ala Lys Lys Leu Lys Asn Thr Leu Leu Ser Leu Asp Ser Gly Glu Ser  
 1265 1270 1275 1280

Met Ser Phe Ser Arg Ser Tyr Gly Gly Gly Val Ser Thr Val Phe Val  
 1285 1290 1295

25 Pro Thr Leu Ser Lys Lys Val Pro Val Pro Val Ile Pro Gly Ala Gly  
 1300 1305 1310

30 Ile Thr Leu Asp Arg Ala Tyr Asn Leu Ser Phe Ser Arg Thr Ser Gly  
 1315 1320 1325

Gly Leu Asn Val Ser Phe Gly Arg Asp Gly Gly Val Ser Gly Asn Ile  
 1330 1335 1340

35 Met Val Ala Thr Gly His Asp Val Met Pro Tyr Met Thr Gly Lys Lys  
 1345 1350 1355 1360

Thr Ser Ala Gly Asn Ala Ser Asp Trp Leu Ser Ala Lys His Lys Ile  
 1365 1370 1375

40 Ser Pro Asp Leu Arg Ile Gly Ala Ala Val Ser Gly Thr Leu Gln Gly  
 1380 1385 1390

Thr Leu Gln Asn Ser Leu Lys Phe Lys Leu Thr Glu Asp Glu Leu Pro  
 1395 1400 1405

Gly Phe Ile His Gly Leu Thr His Gly Thr Leu Thr Pro Ala Glu Leu  
 1410 1415 1420

50 Leu Gln Lys Gly Ile Glu His Gln Met Lys Gln Gly Ser Lys Leu Thr  
 1425 1430 1435 1440

Phe Ser Val Asp Thr Ser Ala Asn Leu Asp Leu Arg Ala Gly Ile Asn  
 1445 1450 1455

55 Leu Asn Glu Asp Gly Ser Lys Pro Asn Gly Val Thr Ala Arg Val Ser  
 1460 1465 1470

60 Ala Gly Leu Ser Ala Ser Ala Asn Leu Ala Ala Gly Ser Arg Glu Arg  
 1475 1480 1485



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	Ser Thr Thr Ser Gly Gln Phe Gly Ser Thr Thr Ser Ala Ser Asn Asn
	1490 1495 1500
5	Arg Pro Thr Phe Leu Asn Gly Val Gly Ala Gly Ala Asn Leu Thr Ala
	1505 1510 1515 1520
	Ala Leu Gly Val Ala His Ser Ser Thr His Glu Gly Lys Pro Val Gly
	1525 1530 1535
10	Ile Phe Pro Ala Phe Thr Ser Thr Asn Val Ser Ala Ala Leu Ala Leu
	1540 1545 1550
	Asp Asn Arg Thr Ser Gln Ser Ile Ser Leu Glu Leu Lys Arg Ala Glu
15	1555 1560 1565
	Pro Val Thr Ser Asn Asp Ile Ser Glu Leu Thr Ser Thr Leu Gly Lys
	1570 1575 1580
20	His Phe Lys Asp Ser Ala Thr Thr Lys Met Leu Ala Ala Leu Lys Glu
	1585 1590 1595 1600
	Leu Asp Asp Ala Lys Pro Ala Glu Gln Leu His Ile Leu Gln Gln His
	1605 1610 1615
25	Phe Ser Ala Lys Asp Val Val Gly Asp Glu Arg Tyr Glu Ala Val Arg
	1620 1625 1630
	Asn Leu Lys Lys Leu Val Ile Arg Gln Gln Ala Ala Asp Ser His Ser
30	1635 1640 1645
	Met Glu Leu Gly Ser Ala Ser His Ser Thr Thr Tyr Asn Asn Leu Ser
	1650 1655 1660
35	Arg Ile Asn Asn Asp Gly Ile Val Glu Leu Leu His Lys His Phe Asp
	1665 1670 1675 1680
	Ala Ala Leu Pro Ala Ser Ser Ala Lys Arg Leu Gly Glu Met Met Asn
	1685 1690 1695
40	Asn Asp Pro Ala Leu Lys Asp Ile Ile Lys Gln Leu Gln Ser Thr Pro
	1700 1705 1710
	Phe Ser Ser Ala Ser Val Ser Met Glu Leu Lys Asp Gly Leu Arg Glu
45	1715 1720 1725
	Gln Thr Glu Lys Ala Ile Leu Asp Gly Lys Val Gly Arg Glu Glu Val
	1730 1735 1740
50	Gly Val Leu Phe Gln Asp Arg Asn Asn Leu Arg Val Lys Ser Val Ser
	1745 1750 1755 1760
	Val Ser Gln Ser Val Ser Lys Ser Glu Gly Phe Asn Thr Pro Ala Leu
	1765 1770 1775
55	Leu Leu Gly Thr Ser Asn Ser Ala Ala Met Ser Met Glu Arg Asn Ile
	1780 1785 1790
	Gly Thr Ile Asn Phe Lys Tyr Gly Gln Asp Gln Asn Thr Pro Arg Arg
60	1795 1800 1805
	Phe Thr Leu Glu Gly Gly Ile Ala Gln Ala Asn Pro Gln Val Ala Ser
	1810 1815 1820

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Ala Leu Thr Asp Leu Lys Lys Glu Gly Leu Glu Met Lys Ser  
 1825 1830 1835

5

This protein or polypeptide is about 198 kDa and has a pI of 8.98.

The present invention relates to an isolated DNA molecule having a nucleotide sequence of SEQ. ID. No. 3 as follows:

10

ATGACATCGT CACAGCAGCG GGTGAAAGG TTTTACAGT ATTTCTCCGC CGGGTGTAAG 60  
 ACGCCCATAC ATCTGAAAGA CGGGGTGTGC GCCCTGTATA ACGAACAAGA TGAGGAGGCG 120  
 15 GCGGTGCTGG AAGTACCGCA ACACAGCGAC AGCCTGTTAC TACACTGCCG AATCATTGAG 180  
 GCTGACCCAC AAACCTCAAT AACCTGTAT TCGATGCTAT TACAGCTGAA TTTTGAAATG 240  
 GCGGCCATGC GCGGCTGTTG GCTGGCGCTG GATGAACTGC ACAACGTGCG TTTATGTTTT 300  
 20 CAGCAGTCGC TGGAGCATCT GGATGAAGCA AGTTTACGCG ATATCGTTAG CGGCTTCATC 360  
 GAACATGCGG CAGAAGTGCG TGAGTATATA GCGCAATTAG ACGAGAGTAG CGCGGCATAA 420

25

This is known as the dspF gene. This isolated DNA molecule of the present invention encodes a hypersensitive response elicitor protein or polypeptide having an amino acid sequence of SEQ. ID. No. 4 as follows:

30

Met Thr Ser Ser Gln Gln Arg Val Glu Arg Phe Leu Gln Tyr Phe Ser  
 1 5 10 15

Ala Gly Cys Lys Thr Pro Ile His Leu Lys Asp Gly Val Cys Ala Leu  
 20 25 30

35

Tyr Asn Glu Gln Asp Glu Glu Ala Ala Val Leu Glu Val Pro Gln His  
 35 40 45

40

Ser Asp Ser Leu Leu Leu His Cys Arg Ile Ile Glu Ala Asp Pro Gln  
 50 55 60

Thr Ser Ile Thr Leu Tyr Ser Met Leu Leu Gln Leu Asn Phe Glu Met  
 65 70 75 80

45

Ala Ala Met Arg Gly Cys Trp Leu Ala Leu Asp Glu Leu His Asn Val  
 85 90 95

Arg Leu Cys Phe Gln Gln Ser Leu Glu His Leu Asp Glu Ala Ser Phe  
 100 105 110

50

Ser Asp Ile Val Ser Gly Phe Ile Glu His Ala Ala Glu Val Arg Glu  
 115 120 125

55

Tyr Ile Ala Gln Leu Asp Glu Ser Ser Ala Ala  
 130 135

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This protein or polypeptide is about 16 kDa and has a pI of 4.45.

Fragments of the above hypersensitive response elicitor polypeptide or protein are encompassed by the present invention.

5           Suitable fragments can be produced by several means. In the first, subclones of the gene encoding the elicitor protein of the present invention are produced by conventional molecular genetic manipulation by subcloning gene fragments. The subclones then are expressed *in vitro* or *in vivo* in bacterial cells to yield a smaller protein or peptide that can be tested for elicitor activity according to  
10 the procedure described below.

As an alternative, fragments of an elicitor protein can be produced by digestion of a full-length elicitor protein with proteolytic enzymes like chymotrypsin or *Staphylococcus* proteinase A, or trypsin. Different proteolytic enzymes are likely to cleave elicitor proteins at different sites based on the amino acid sequence of the  
15 elicitor protein. Some of the fragments that result from proteolysis may be active elicitors of resistance.

In another approach, based on knowledge of the primary structure of the protein, fragments of the elicitor protein gene may be synthesized by using the PCR technique together with specific sets of primers chosen to represent particular  
20 portions of the protein. These then would be cloned into an appropriate vector for increased expression of a truncated peptide or protein.

Chemical synthesis can also be used to make suitable fragments. Such a synthesis is carried out using known amino acid sequences for the elicitor being produced. Alternatively, subjecting a full length elicitor to high temperatures and  
25 pressures will produce fragments. These fragments can then be separated by conventional procedures (e.g., chromatography, SDS-PAGE).

Variants may also (or alternatively) be modified by, for example, the deletion or addition of amino acids that have minimal influence on the properties, secondary structure and hydrophobic nature of the polypeptide. For example, a  
30 polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification, or identification of the polypeptide.

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Suitable DNA molecules are those that hybridize to a DNA molecule comprising a nucleotide sequence of SEQ. ID. Nos. 1 and 3, under stringent conditions. An example of suitable high stringency conditions is when hybridization is carried out at 65°C for 20 hours in a medium containing 1M NaCl, 50 mM Tris-HCl, pH 7.4, 10 mM EDTA, 0.1% sodium dodecyl sulfate, 0.2% ficoll, 0.2%  
5 polyvinylpyrrolidone, 0.2% bovine serum albumin, 50 µm g/ml *E. coli* DNA. However, any DNA molecules hybridizing to a DNA molecule comprising the nucleotide sequences of SEQ. ID. Nos. 1 and 3, under such stringent conditions must not be identical to the nucleic acids encoding the hypersensitive response elicitor  
10 proteins or polypeptides of *E. amylovora* (as disclosed by Wei, Z.-M., et al., "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992), which is hereby incorporated by reference), *Erwinia chrysanthemi* (as disclosed by Bauer, et. al., "*Erwinia chrysanthemi* Harpin<sub>Ech</sub>: Soft-Rot Pathogenesis," MPMI 8(4): 484-91 (1995), which is hereby  
15 incorporated by reference), *Erwinia carotovora* (as disclosed by Cui, et. al., "The RsmA<sup>-</sup> Mutants of *Erwinia carotovora* subsp. *carotovora* Strain Ecc71 Overexpress *hrpN*<sub>Ecc</sub> and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves," MPMI 9(7): 565-73 (1966), which is hereby incorporated by reference), *Erwinia stewartii* (as disclosed by Ahmad, et. al., "Harpin is not Necessary for the  
20 Pathogenicity of *Erwinia stewartii* on Maize," 8th Int'l. Cong. Molec. Plant-Microb. Inter. July 14-19, 1996 and Ahmad, et. al., "Harpin is not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," Ann. Mtg. Am. Phytopath. Soc. July 27-31, 1996), which are hereby incorporated by reference), and *Pseudomonas syringae* pv. *syringae* (WO 94/26782 to Cornell Research Foundation, Inc., which is  
25 hereby incorporated by reference).

The protein or polypeptide of the present invention is preferably produced in purified form (preferably at least about 80%, more preferably 90%, pure) by conventional techniques. Typically, the protein or polypeptide of the present invention is secreted into the growth medium of recombinant host cells.  
30 Alternatively, the protein or polypeptide of the present invention is produced but not secreted into growth medium. In such cases, to isolate the protein, the host cell (e.g., *E. coli*) carrying a recombinant plasmid is propagated, lysed by sonication, heat, or

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chemical treatment, and the homogenate is centrifuged to remove bacterial debris.

The supernatant is then subjected to sequential ammonium sulfate precipitation. The fraction containing the polypeptide or protein of the present invention is subjected to gel filtration in an appropriately sized dextran or polyacrylamide column to separate the proteins. If necessary, the protein fraction may be further purified by HPLC.

The DNA molecule encoding the hypersensitive response elicitor polypeptide or protein can be incorporated in cells using conventional recombinant DNA technology. Generally, this involves inserting the DNA molecule into an expression system to which the DNA molecule is heterologous (i.e. not normally present). The heterologous DNA molecule is inserted into the expression system or vector in proper sense orientation and correct reading frame. The vector contains the necessary elements for the transcription and translation of the inserted protein-coding sequences.

U.S. Patent No. 4,237,224 to Cohen and Boyer, which is hereby incorporated by reference, describes the production of expression systems in the form of recombinant plasmids using restriction enzyme cleavage and ligation with DNA ligase. These recombinant plasmids are then introduced by means of transformation and replicated in unicellular cultures including procaryotic organisms and eucaryotic cells grown in tissue culture.

Recombinant genes may also be introduced into viruses, such as vaccinia virus. Recombinant viruses can be generated by transfection of plasmids into cells infected with virus.

Suitable vectors include, but are not limited to, the following viral vectors such as lambda vector system gt11, gt WES.tB, Charon 4, and plasmid vectors such as pBR322, pBR325, pACYC177, pACYC1084, pUC8, pUC9, pUC18, pUC19, pLG339, pR290, pKC37, pKC101, SV 40, pBluescript II SK +/- or KS +/- (see "Stratagene Cloning Systems" Catalog (1993) from Stratagene, La Jolla, Calif, which is hereby incorporated by reference), pQE, pIH821, pGEX, pET series (see F.W. Studier et. al., "Use of T7 RNA Polymerase to Direct Expression of Cloned Genes," Gene Expression Technology vol. 185 (1990), which is hereby incorporated by reference), and any derivatives thereof. Recombinant molecules can be introduced into cells via transformation, particularly transduction, conjugation, mobilization, or

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electroporation. The DNA sequences are cloned into the vector using standard cloning procedures in the art, as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York (1989), which is hereby incorporated by reference.

5                   A variety of host-vector systems may be utilized to express the protein-encoding sequence(s). Primarily, the vector system must be compatible with the host cell used. Host-vector systems include but are not limited to the following: bacteria transformed with bacteriophage DNA, plasmid DNA, or cosmid DNA; microorganisms such as yeast containing yeast vectors; mammalian cell systems  
10                   infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); and plant cells infected by bacteria. The expression elements of these vectors vary in their strength and specificities. Depending upon the host-vector system utilized, any one of a number of suitable transcription and translation elements can be used.

15                   Different genetic signals and processing events control many levels of gene expression (e.g., DNA transcription and messenger RNA (mRNA) translation).

                  Transcription of DNA is dependent upon the presence of a promotor which is a DNA sequence that directs the binding of RNA polymerase and thereby promotes mRNA synthesis. The DNA sequences of eucaryotic promoters differ from  
20                   those of procaryotic promoters. Furthermore, eucaryotic promoters and accompanying genetic signals may not be recognized in or may not function in a procaryotic system, and, further, procaryotic promoters are not recognized and do not function in eucaryotic cells.

                  Similarly, translation of mRNA in procaryotes depends upon  
25                   the presence of the proper procaryotic signals which differ from those of eucaryotes. Efficient translation of mRNA in procaryotes requires a ribosome binding site called the Shine-Dalgarno ("SD") sequence on the mRNA. This sequence is a short nucleotide sequence of mRNA that is located before the start codon, usually AUG, which encodes the amino-terminal methionine of the protein. The SD sequences are  
30                   complementary to the 3'-end of the 16S rRNA (ribosomal RNA) and probably promote binding of mRNA to ribosomes by duplexing with the rRNA to allow correct positioning of the ribosome. For a review on maximizing gene expression, see

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Roberts and Lauer, Methods in Enzymology, 68:473 (1979), which is hereby incorporated by reference.

Promoters vary in their "strength" (i.e. their ability to promote transcription). For the purposes of expressing a cloned gene, it is desirable to use strong promoters in order to obtain a high level of transcription and, hence, expression of the gene. Depending upon the host cell system utilized, any one of a number of suitable promoters may be used. For instance, when cloning in *E. coli*, its bacteriophages, or plasmids, promoters such as the T7 phage promoter, *lac* promoter, *trp* promoter, *recA* promoter, ribosomal RNA promoter, the P<sub>R</sub> and P<sub>L</sub> promoters of coliphage lambda and others, including but not limited, to *lacUV5*, *ompF*, *bla*, *lpp*, and the like, may be used to direct high levels of transcription of adjacent DNA segments. Additionally, a hybrid *trp-lacUV5 (tac)* promoter or other *E. coli* promoters produced by recombinant DNA or other synthetic DNA techniques may be used to provide for transcription of the inserted gene.

Bacterial host cell strains and expression vectors may be chosen which inhibit the action of the promoter unless specifically induced. In certain operations, the addition of specific inducers is necessary for efficient transcription of the inserted DNA. For example, the *lac* operon is induced by the addition of lactose or IPTG (isopropylthio-beta-D-galactoside). A variety of other operons, such as *trp*, *pro*, etc., are under different controls.

Specific initiation signals are also required for efficient gene transcription and translation in procaryotic cells. These transcription and translation initiation signals may vary in "strength" as measured by the quantity of gene specific messenger RNA and protein synthesized, respectively. The DNA expression vector, which contains a promoter, may also contain any combination of various "strong" transcription and/or translation initiation signals. For instance, efficient translation in *E. coli* requires an SD sequence about 7-9 bases 5' to the initiation codon ("ATG") to provide a ribosome binding site. Thus, any SD-ATG combination that can be utilized by host cell ribosomes may be employed. Such combinations include but are not limited to the SD-ATG combination from the *cro* gene or the *N* gene of coliphage lambda, or from the *E. coli* tryptophan E, D, C, B or A genes. Additionally, any SD-

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ATG combination produced by recombinant DNA or other techniques involving incorporation of synthetic nucleotides may be used.

Once the isolated DNA molecule encoding the hypersensitive response elicitor polypeptide or protein has been cloned into an expression system, it is ready  
5 to be incorporated into a host cell. Such incorporation can be carried out by the various forms of transformation noted above, depending upon the vector/host cell system. Suitable host cells include, but are not limited to, bacteria, virus, yeast, mammalian cells, insect, plant, and the like.

The present invention further relates to methods of imparting disease  
10 resistance to plants, enhancing plant growth, and/or effecting insect control for plants. These methods involve applying a hypersensitive response elicitor polypeptide or protein in a non-infectious form to all or part of a plant or a plant seed under conditions where the polypeptide or protein contacts all or part of the cells of the plant or plant seed. Alternatively, the hypersensitive response elicitor protein or  
15 polypeptide can be applied to plants such that seeds recovered from such plants themselves are able to impart disease resistance in plants, to enhance plant growth, and/or to effect insect control.

As an alternative to applying a hypersensitive response elicitor polypeptide or protein to plants or plant seeds in order to impart disease resistance in  
20 plants, to effect plant growth, and/or to control insects on the plants or plants grown from the seeds, transgenic plants or plant seeds can be utilized. When utilizing transgenic plants, this involves providing a transgenic plant transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and growing the plant under conditions effective to permit that DNA molecule to impart  
25 disease resistance to plants, to enhance plant growth, and/or to control insects. Alternatively, a transgenic plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein can be provided and planted in soil. A plant is then propagated from the planted seed under conditions effective to permit that DNA molecule to impart disease resistance to plants, to enhance plant  
30 growth, and/or to control insects.

The embodiment of the present invention where the hypersensitive response elicitor polypeptide or protein is applied to the plant or plant seed can be



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carried out in a number of ways, including: 1) application of an isolated elicitor polypeptide or protein; 2) application of bacteria which do not cause disease and are transformed with genes encoding a hypersensitive response elicitor polypeptide or protein; and 3) application of bacteria which cause disease in some plant species (but not in those to which they are applied) and naturally contain a gene encoding the hypersensitive response elicitor polypeptide or protein.

In one embodiment of the present invention, the hypersensitive response elicitor polypeptide or protein of the present invention can be isolated from *Erwinia amylovora* as described in the Examples *infra*. Preferably, however, the isolated hypersensitive response elicitor polypeptide or protein of the present invention is produced recombinantly and purified as described *supra*.

In other embodiments of the present invention, the hypersensitive response elicitor polypeptide or protein of the present invention can be applied to plants or plant seeds by applying bacteria containing genes encoding the hypersensitive response elicitor polypeptide or protein. Such bacteria must be capable of secreting or exporting the polypeptide or protein so that the elicitor can contact plant or plant seed cells. In these embodiments, the hypersensitive response elicitor polypeptide or protein is produced by the bacteria *in planta* or on seeds or just prior to introduction of the bacteria to the plants or plant seeds.

In one embodiment of the bacterial application mode of the present invention, the bacteria do not cause the disease and have been transformed (e.g., recombinantly) with genes encoding a hypersensitive response elicitor polypeptide or protein. For example, *E. coli*, which does not elicit a hypersensitive response in plants, can be transformed with genes encoding a hypersensitive response elicitor polypeptide or protein and then applied to plants. Bacterial species other than *E. coli* can also be used in this embodiment of the present invention.

In another embodiment of the bacterial application mode of the present invention, the bacteria do cause disease and naturally contain a gene encoding a hypersensitive response elicitor polypeptide or protein. Examples of such bacteria are noted above. However, in this embodiment, these bacteria are applied to plants or their seeds which are not susceptible to the disease carried by the bacteria. For example, *Erwinia amylovora* causes disease in apple or pear but not in tomato.

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However, such bacteria will elicit a hypersensitive response in tomato. Accordingly, in accordance with this embodiment of the present invention, *Erwinia amylovora* can be applied to tomato plants or seeds to enhance growth without causing disease in that species.

5                   The method of the present invention can be utilized to treat a wide variety of plants or their seeds to impart disease resistance, enhance growth, and/or control insects. Suitable plants include dicots and monocots. More particularly, useful crop plants can include: alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage,  
10 brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane. Examples of suitable ornamental plants are:  
*Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum,  
15 carnation, and zinnia.

                  With regard to the use of the hypersensitive response elicitor protein or polypeptide of the present invention in imparting disease resistance, absolute immunity against infection may not be conferred, but the severity of the disease is reduced and symptom development is delayed. Lesion number, lesion size, and  
20 extent of sporulation of fungal pathogens are all decreased. This method of imparting disease resistance has the potential for treating previously untreatable diseases, treating diseases systemically which might not be treated separately due to cost, and avoiding the use of infectious agents or environmentally harmful materials.

                  The method of imparting pathogen resistance to plants in accordance  
25 with the present invention is useful in imparting resistance to a wide variety of pathogens including viruses, bacteria, and fungi. Resistance, *inter alia*, to the following viruses can be achieved by the method of the present invention: *Tobacco mosaic virus* and *Tomato mosaic virus*. Resistance, *inter alia*, to the following bacteria can also be imparted to plants in accordance with present invention:  
30 *Pseudomonas solanacearum*, *Pseudomonas syringae* pv. *tabaci*, and *Xanthomonas campestris* pv. *pelargonii*. Plants can be made resistant, *inter alia*, to the following

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fungi by use of the method of the present invention: *Fusarium oxysporum* and *Phytophthora infestans*.

With regard to the use of the hypersensitive response elicitor protein or polypeptide of the present invention to enhance plant growth, various forms of plant growth enhancement or promotion can be achieved. This can occur as early as when plant growth begins from seeds or later in the life of a plant. For example, plant growth according to the present invention encompasses greater yield, increased quantity of seeds produced, increased percentage of seeds germinated, increased plant size, greater biomass, more and bigger fruit, earlier fruit coloration, and earlier fruit and plant maturation. As a result, the present invention provides significant economic benefit to growers. For example, early germination and early maturation permit crops to be grown in areas where short growing seasons would otherwise preclude their growth in that locale. Increased percentage of seed germination results in improved crop stands and more efficient seed use. Greater yield, increased size, and enhanced biomass production allow greater revenue generation from a given plot of land.

Another aspect of the present invention is directed to effecting any form of insect control for plants. For example, insect control according to the present invention encompasses preventing insects from contacting plants to which the hypersensitive response elicitor has been applied, preventing direct insect damage to plants by feeding injury, causing insects to depart from such plants, killing insects proximate to such plants, interfering with insect larval feeding on such plants, preventing insects from colonizing host plants, preventing colonizing insects from releasing phytotoxins, etc. The present invention also prevents subsequent disease damage to plants resulting from insect infection.

The present invention is effective against a wide variety of insects. European corn borer is a major pest of corn (dent and sweet corn) but also feeds on over 200 plant species including green, wax, and lima beans and edible soybeans, peppers, potato, and tomato plus many weed species. Additional insect larval feeding pests which damage a wide variety of vegetable crops include the following: beet armyworm, cabbage looper, corn ear worm, fall armyworm, diamondback moth, cabbage root maggot, onion maggot, seed corn maggot, pickleworm (melonworm), pepper maggot, and tomato pinworm. Collectively, this group of insect pests

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represents the most economically important group of pests for vegetable production worldwide.

The method of the present invention involving application of the hypersensitive response elicitor polypeptide or protein can be carried out through a variety of procedures when all or part of the plant is treated, including leaves, stems, roots, propagules (e.g., cuttings), etc. This may (but need not) involve infiltration of the hypersensitive response elicitor polypeptide or protein into the plant. Suitable application methods include high or low pressure spraying, injection, and leaf abrasion proximate to when elicitor application takes place. When treating plant seeds, in accordance with the application embodiment of the present invention, the hypersensitive response elicitor protein or polypeptide can be applied by low or high pressure spraying, coating, immersion, or injection. Other suitable application procedures can be envisioned by those skilled in the art provided they are able to effect contact of the hypersensitive response elicitor polypeptide or protein with cells of the plant or plant seed. Once treated with the hypersensitive response elicitor of the present invention, the seeds can be planted in natural or artificial soil and cultivated using conventional procedures to produce plants. After plants have been propagated from seeds treated in accordance with the present invention, the plants may be treated with one or more applications of the hypersensitive response elicitor protein or polypeptide to impart disease resistance to plants, to enhance plant growth, and/or to control insects on the plants.

The hypersensitive response elicitor polypeptide or protein can be applied to plants or plant seeds in accordance with the present invention alone or in a mixture with other materials. Alternatively, the hypersensitive response elicitor polypeptide or protein can be applied separately to plants with other materials being applied at different times.

A composition suitable for treating plants or plant seeds in accordance with the application embodiment of the present invention contains a hypersensitive response elicitor polypeptide or protein in a carrier. Suitable carriers include water, aqueous solutions, slurries, or dry powders. In this embodiment, the composition contains greater than 500 nM hypersensitive response elicitor polypeptide or protein.

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Although not required, this composition may contain additional additives including fertilizer, insecticide, fungicide, nematocide, and mixtures thereof. Suitable fertilizers include  $(\text{NH}_4)_2\text{NO}_3$ . An example of a suitable insecticide is Malathion. Useful fungicides include Captan.

5                   Other suitable additives include buffering agents, wetting agents, coating agents, and abrading agents. These materials can be used to facilitate the process of the present invention. In addition, the hypersensitive response elicitor polypeptide or protein can be applied to plant seeds with other conventional seed formulation and treatment materials, including clays and polysaccharides.

10                   In the alternative embodiment of the present invention involving the use of transgenic plants and transgenic seeds, a hypersensitive response elicitor polypeptide or protein need not be applied topically to the plants or seeds. Instead, transgenic plants transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein are produced according to procedures well  
15 known in the art.

The vector described above can be microinjected directly into plant cells by use of micropipettes to transfer mechanically the recombinant DNA. Crossway, Mol. Gen. Genetics, 202:179-85 (1985), which is hereby incorporated by reference. The genetic material may also be transferred into the plant cell using  
20 polyethylene glycol. Krens, et al., Nature, 296:72-74 (1982), which is hereby incorporated by reference.

Another approach to transforming plant cells with a gene which imparts resistance to pathogens is particle bombardment (also known as biolistic transformation) of the host cell. This can be accomplished in one of several ways.  
25 The first involves propelling inert or biologically active particles at cells. This technique is disclosed in U.S. Patent Nos. 4,945,050, 5,036,006, and 5,100,792, all to Sanford et al., which are hereby incorporated by reference. Generally, this procedure involves propelling inert or biologically active particles at the cells under conditions effective to penetrate the outer surface of the cell and to be incorporated within the  
30 interior thereof. When inert particles are utilized, the vector can be introduced into the cell by coating the particles with the vector containing the heterologous DNA. Alternatively, the target cell can be surrounded by the vector so that the vector is

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carried into the cell by the wake of the particle. Biologically active particles (e.g., dried bacterial cells containing the vector and heterologous DNA) can also be propelled into plant cells.

Yet another method of introduction is fusion of protoplasts with other entities, either minicells, cells, lysosomes or other fusible lipid-surfaced bodies. Fraley, et al., Proc. Natl. Acad. Sci. USA, 79:1859-63 (1982), which is hereby incorporated by reference.

The DNA molecule may also be introduced into the plant cells by electroporation. Fromm et al., Proc. Natl. Acad. Sci. USA, 82:5824 (1985), which is hereby incorporated by reference. In this technique, plant protoplasts are electroporated in the presence of plasmids containing the expression cassette. Electrical impulses of high field strength reversibly permeabilize biomembranes allowing the introduction of the plasmids. Electroporated plant protoplasts reform the cell wall, divide, and regenerate.

Another method of introducing the DNA molecule into plant cells is to infect a plant cell with *Agrobacterium tumefaciens* or *A. rhizogenes* previously transformed with the gene. Under appropriate conditions known in the art, the transformed plant cells are grown to form shoots or roots, and develop further into plants. Generally, this procedure involves inoculating the plant tissue with a suspension of bacteria and incubating the tissue for 48 to 72 hours on regeneration medium without antibiotics at 25-28°C.

*Agrobacterium* is a representative genus of the gram-negative family Rhizobiaceae. Its species are responsible for crown gall (*A. tumefaciens*) and hairy root disease (*A. rhizogenes*). The plant cells in crown gall tumors and hairy roots are induced to produce amino acid derivatives known as opines, which are catabolized only by the bacteria. The bacterial genes responsible for expression of opines are a convenient source of control elements for chimeric expression cassettes. In addition, assaying for the presence of opines can be used to identify transformed tissue.

Heterologous genetic sequences can be introduced into appropriate plant cells, by means of the Ti plasmid of *A. tumefaciens* or the Ri plasmid of *A. rhizogenes*. The Ti or Ri plasmid is transmitted to plant cells on infection by

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Agrobacterium and is stably integrated into the plant genome. J. Schell, Science, 237:1176-83 (1987), which is hereby incorporated by reference.

After transformation, the transformed plant cells must be regenerated.

Plant regeneration from cultured protoplasts is described in Evans et al., Handbook of Plant Cell Cultures, Vol. 1: (MacMillan Publishing Co., New York, 1983); and Vasil I.R. (ed.), Cell Culture and Somatic Cell Genetics of Plants, Acad. Press, Orlando, Vol. I, 1984, and Vol. III (1986), which are hereby incorporated by reference.

It is known that practically all plants can be regenerated from cultured cells or tissues, including but not limited to, all major species of sugarcane, sugar beets, cotton, fruit trees, and legumes.

Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts or a petri plate containing transformed explants is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, embryo formation can be induced in the callus tissue. These embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is usually reproducible and repeatable.

After the expression cassette is stably incorporated in transgenic plants, it can be transferred to other plants by sexual crossing. Any of a number of standard breeding techniques can be used, depending upon the species to be crossed.

Once transgenic plants of this type are produced, the plants themselves can be cultivated in accordance with conventional procedure with the presence of the gene encoding the hypersensitive response elicitor resulting in disease resistance, enhanced plant growth, and/or control of insects on the plant. Alternatively, transgenic seeds are recovered from the transgenic plants. These seeds can then be planted in the soil and cultivated using conventional procedures to produce transgenic plants. The transgenic plants are propagated from the planted transgenic seeds under

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conditions effective to impart disease resistance to plants, to enhance plant growth, and/or to control insects. While not wishing to be bound by theory, such disease resistance, growth enhancement, and/or insect control may be RNA mediated or may result from expression of the elicitor polypeptide or protein.

5                   When transgenic plants and plant seeds are used in accordance with the present invention, they additionally can be treated with the same materials as are used to treat the plants and seeds to which a hypersensitive response elicitor polypeptide or protein is applied. These other materials, including hypersensitive response elicitors, can be applied to the transgenic plants and plant seeds by the above-noted procedures, including high or low pressure spraying, injection, coating, and immersion. Similarly, after plants have been propagated from the transgenic plant seeds, the plants may be treated with one or more applications of the hypersensitive response elicitor to impart disease resistance, enhance growth, and/or control insects. Such plants may also be treated with conventional plant treatment agents (e.g., insecticides, fertilizers, etc.).

15                   Another aspect of the present invention is to utilize the subject elicitor proteins or polypeptides to design molecules that will inactivate, destroy, or bind to these proteins or polypeptides. Since these elicitors are found in plant pathogens, particularly *Erwinia amylovora*, the pathogens themselves can be neutralized by the designed molecules so that disease and/or hypersensitive response is prevented or altered. Examples of disease preventing molecules are antibodies, such as

20                   monoclonal or polyclonal antibodies, raised against the elicitor proteins or polypeptides of the present invention or binding portions thereof. Other examples of disease preventing molecules include antibody fragments, half-antibodies, hybrid derivatives, probes, and other molecular constructs.

25                   Monoclonal antibody production may be effected by techniques which are well-known in the art. Basically, the process involves first obtaining immune cells (lymphocytes) from the spleen of a mammal (e.g., mouse) which has been previously immunized, either *in vivo* or *in vitro*, with the antigen of interest (e.g., an elicitor protein or polypeptide of the present invention or binding portions thereof).

30                   The antibody-secreting lymphocytes are then fused with (mouse) myeloma cells or transformed cells, which are capable of replicating indefinitely in cell culture, thereby producing an immortal, immunoglobulin-secreting cell line. The resulting fused cells,



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or hybridomas, are cultured, and the resulting colonies screened for the production of the desired monoclonal antibodies. Colonies producing such antibodies are cloned, and grown either *in vivo* or *in vitro* to produce large quantities of antibody. A description of the theoretical basis and practical methodology of fusing such cells is set forth in Kohler and Milstein, Nature 256:495 (1975), which is hereby incorporated by reference.

Mammalian lymphocytes are immunized by *in vivo* immunization of the animal (e.g., a mouse) with the elicitor proteins or polypeptides of the present invention or binding portions thereof. Such immunizations are repeated as necessary at intervals of up to several weeks to obtain a sufficient titer of antibodies. Following the last antigen boost, the animals are sacrificed and spleen cells removed.

Fusion with mammalian myeloma cells or other fusion partners capable of replicating indefinitely in cell culture is effected by standard and well-known techniques, for example, by using polyethylene glycol ("PEG") or other fusing agents (See Milstein and Kohler, Eur. J. Immunol. 6:511 (1976), which is hereby incorporated by reference). This immortal cell line, which is preferably murine, but may also be derived from cells of other mammalian species, including but not limited to rats, is selected to be deficient in enzymes necessary for the utilization of certain nutrients, to be capable of rapid growth, and to have good fusion capability. Many such cell lines are known to those skilled in the art, and others are regularly described.

Procedures for raising polyclonal antibodies are also well known. Typically, such antibodies can be raised by administering the elicitor proteins or polypeptides of the present invention or binding portions thereof subcutaneously to New Zealand white rabbits which have first been bled to obtain pre-immune serum. The antigens can be injected at a total volume of 100  $\mu$ l per site at six different sites. Each injected material will contain synthetic surfactant adjuvant pluronic polyols, or pulverized acrylamide gel containing the protein or polypeptide after SDS-polyacrylamide gel electrophoresis. The rabbits are then bled two weeks after the first injection and periodically boosted with the same antigen three times every six weeks. A sample of serum is then collected 10 days after each boost. Polyclonal antibodies are then recovered from the serum by affinity chromatography using the

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corresponding antigen to capture the antibody. Ultimately, the rabbits are euthenized with pentobarbital 150 mg/Kg IV. This and other procedures for raising polyclonal antibodies are disclosed in E. Harlow, et. al., editors, Antibodies: A Laboratory Manual (1988), which is hereby incorporated by reference.

5 In addition to utilizing whole antibodies, the processes of the present invention encompass use of binding portions of such antibodies. Such binding portions include Fab fragments, F(ab')<sub>2</sub> fragments, and Fv fragments. These antibody fragments can be made by conventional procedures, such as proteolytic fragmentation procedures, as described in J. Goding, Monoclonal Antibodies: Principles and  
10 Practice, pp. 98-118 (N.Y. Academic Press 1983), which is hereby incorporated by reference.

Alternatively, the processes of the present invention can utilize probes or ligands found either in nature or prepared synthetically by recombinant DNA procedures or other biological or molecular procedures. Suitable probes or ligands  
15 are molecules which bind to the elicitor proteins or polypeptides of the present invention or binding portions thereof.

Avirulence (*avr*) genes (see Vivian, A., et al, Microbiology, 143:693-704 (1997); Leach, J. E., et al., Annu. Rev. Phytopathol., 34:153-179 (1996); Dangl, J. L. "Bacterial Pathogenesis of Plants and Animals: Molecular and Cellular  
20 Mechanisms," in Current Topics in Microbiology and Immunology, Dangl, J. L., ed. (Springer, Berlin), Vol. 192, pp. 99-118 (1994), which are hereby incorporated by reference) generate signals that trigger defense responses leading to disease resistance in plants with corresponding resistance (*R*) genes. Typically, *avr* genes are isolated by expressing a cosmid library from one pathogen in another pathogen and screening  
25 for narrowed host range. *avr* genes traditionally have been considered as negative determinants of host specificity at the race-cultivar level, but some, including the *avrE* locus from the bacterial speck pathogen *Pseudomonas syringae* pathovar (pv.) tomato (Kobayashi, D. Y., et al., Proc. Natl. Acad. Sci. USA, 86:157-61 (1989), which is hereby incorporated by reference), restrict host range at the pathovar-species or  
30 species-species level (Whalen, M. C., et al., Proc. Natl. Acad. Sci. USA, 85:6743-47 (1988); Swarup, S., et al., Mol. Plant-Microbe Interact., 5:204-13 (1992), which are hereby incorporated by reference). Many *avr* genes, including *avrE*, are Hrp

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regulated. *avrE* and *avrPphE* (Mansfield, J., et al., Mol. Plant-Microbe Interact., 7:726-39 (1994), which is hereby incorporated by reference) are physically linked to *hrp* genes.

When expressed *in trans*, the *avrE* locus renders *P. syringae* pv. glycinea, which causes bacterial blight of soybean, avirulent in all cultivars (Lorang, J. M., et al., Mol. Plant-Microbe Interact., 8:49-57 (1995), which is hereby incorporated by reference). The locus comprises two convergent transcription units, one preceded by a putative  $\sigma^{54}$  promoter and the other by a *hrp* box, a sequence found upstream of many *hrp* and *avr* genes that are positively regulated by the alternate sigma factor HrpL (Innes, R. W., et al., J. Bacteriol., 175:4859-69 (1993); Shen, H., et al., J. Bacteriol., 175:5916-24 (1993); Xiao, Y., et al., J. Bacteriol., 176:3089-91 (1994), which are hereby incorporated by reference). Expression of both transcripts require HrpL. The *avrE* locus contributes quantitatively to the virulence in tomato leaves of *P. syringae* pv. tomato strain PT23, but not of strain DC3000 (Lorang, J. M., et al., Mol. Plant-Microbe Interact., 8:49-57 (1995); Lorang, J. M., et al., Mol. Plant-Microbe Interact., 7:508-515 (1994)).

Thus, *avr* genes in plant pathogens bind to disease resistance genes in plants which are not susceptible to that pathogen. In view of the homology of the DNA molecules of the present invention to *avr* genes in plant pathogens, these DNA molecules can be used to identify corresponding plant disease resistance genes. Such identification is carried out by traditional plant breeding techniques in which a pathogen carrying the *avr* gene is inoculated to plants in screening to track inheritance or identify disruption of the resistance. Once identified, the resistance gene can be isolated by either of two approaches that have proved successful in recent years (see Staskawicz et al., Science, 68:661-67 (1995)). These are positional or map-based cloning and insertional mutagenesis or transposon tagging. Because there may be no *DspE*-insensitive cultivars (susceptible to *Pseudomonas* harboring *dspE*; each of four soybean cultivars tested responded to *dspE*), map-based cloning (which requires crosses between susceptible and resistant lines to identify the position of the resistance gene relative to other genes) may not be feasible. The preferred approach would more likely involve insertional mutagenesis, using the *dspE* gene or protein in

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screens to identify lines which had lost the the product of dspE due to transposon tagging of the corresponding resistance gene.

### **EXAMPLES**

5

#### **Example 1 - Recombinant DNA techniques.**

Isolation of DNA, restriction enzyme digests, ligation, transformation of *Escherichia coli*, and construction of and colony hybridization to screen a *P. syringae* pv. tomato DC3000 genomic library were performed as described by Sambrook, et al. (Sambrook, J., et al., Molecular cloning: A Laboratory manual, (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY) (1989), which is hereby incorporated by reference). The library was constructed using pCPP47 (Bauer, D. W., et al., Mol. Plant-Microbe Interact., 10:369-379 (1997), which is hereby incorporated by reference). Except where noted, *E. coli* DH5 and *E. coli* DH5 $\alpha$  were used as hosts for DNA clones, and pBluescript or pBC plasmids (Stratagene, La Jolla, CA) were used as vectors. *E. amylovora* was transformed by electroporation as described (Bauer, D. W. in "Molecular Genetics of Pathogenicity of *Erwinia amylovora*: Techniques, Tools and Their Applications", (Ph. D. Thesis), Cornell University, Ithaca, NY (1990), which is hereby incorporated by reference). Plasmids were mobilized into *E. amylovora* and *P. syringae* using pRK2013 (Figurski, D., et al., Proc. Natl. Acad. Sci. USA 76:1648-1652 (1979), which is hereby incorporated by reference).

#### **Example 2 - Nucleotide sequencing and analysis.**

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The nucleotide sequence of the dsp region of *E. amylovora* strain Ea321 was determined using subclones of pCPP430 (Beer, S. V., et al., in Advances in Molecular Genetics of Plant-Microbe Interactions, Hennecke, H., et al., eds. (Kluwer Academic Publishers, Dordrecht, The Netherlands), pp. 53-60 (1991), which is hereby incorporated by reference). The nucleotide sequence of the *avrE* locus was determined using subclones of pCPP2357, a clone selected from a *P. syringae* pv. tomato DC3000 genomic cosmid library based on hybridization with the *hrpRS* operon of *P. syringae* pv. syringae, and the finding, based on partial sequencing, that it contained the *avrE* locus. Nucleotide sequencing was performed by the Cornell

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Biotechnology Sequencing Facility on a Model 377 Sequencer (Perkin Elmer/Applied Biosystems Division, Foster City, CA). Sequence assembly, analysis, and comparisons were performed using the programs of the GCG software package, version 7.1 (Genetics Computer Groups, Inc., Madison, WI) and DNASTAR (DNASTAR, Inc., Madison, WI). Database searches were performed using BLAST (Altschul, S. F., et al., Proc. Nat. Acad. Sci. USA, 87:5509-5513 (1990) which is hereby incorporated by reference).

**Example 3 - Expression of DspE and DspE' in *E. coli***

10           The dspE operon was cloned in two pieces into pCPP50, a derivative of PINIII<sup>113</sup>-A2 (Duffaud, G. D., et al. in Methods in Enzymology, Wu, R., et al., eds. (Academic Press, New York), 153:492-50 (1987), which is hereby incorporated by reference) with an expanded polylinker, yielding pCPP1259. Expression in pCPP1259 is driven by the *Ipp* promoter of *E. coli*, under the control of the *lac* operator. An intermediate clone, pCPP1244, extending from the start of the operon to the *Bam*HI site in the middle of *dspE*, also was isolated. *E. coli* DH5 $\alpha$  strains containing pCPP1259 and pCPP1244 were grown in LB at 37°C to an OD<sub>620</sub> of 0.3. Isopropylthio- $\beta$ -D-galactoside then was added to 1 mM, and the cells further incubated until reaching an OD<sub>620</sub> of 0.5. Cells were concentrated two-fold, lysed and subjected to SDS-PAGE as previously described (Sambrook, J., et al., Molecular cloning: A Laboratory Manual (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY) (1989), which is hereby incorporated by reference), using 7.5% acrylamide. Cells containing pCPP50 were included for comparison. Proteins were visualized by Coomassie staining.

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**Example 4 - Deletion mutagenesis of *dspE***

1554 bp were deleted from the 5' *Hind*III-*Bam*HI fragment of *dspE* in pCPP1237 using unique *Stu*I and *Sma*I sites. The mutagenized clone then was inserted into the suicide vector pKNG101 (Kaniga, K., et al., Gene, 109:137-42 (1991), which is hereby incorporated by reference) using *E. coli* SM10 $\lambda$ pir as a host, yielding pCPP1241. The mutation, designated  $\Delta$ 1554, then was transferred into *E. amylovora* strains using marker eviction as described previously (Bogdanove, A. J.,

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et al., *J. Bacteriol.*, 178:1720-30 (1996), which is hereby incorporated by reference). 1521 bp were deleted from the 3' *HindIII* fragment of *dspE* in pCPP1246 using two *BstEII* sites blunted with Klenow fragment. This mutation,  $\Delta 1521$ , was transferred into *E. amylovora* strains as above.

5

**Example 5 - Pathogenicity assays.**

For *E. amylovora* strains, cell suspensions of  $5 \times 10^8$  colony-forming units (cfu) per ml were pipetted into wells cut in immature Bartlett pear fruit, or stabbed into Jonamac apple and cotoneaster shoots, and assays carried out as described previously (Beer, S. V., in *Methods in Phytobacteriology*, Klement, Z., et al., eds. (Adadémiai Kiadó, Budapest), pp. 373-374 (the "1990"); Aldwinckle, H. S., et al., *Phytopathology*, 66:1439-44 (1976), which are hereby incorporated by reference). For *P. syringae* pv. *glycinea*, panels of primary leaves of 2-week-old soybean seedlings (*Glycine max*, cultivar Norchief) were infiltrated with bacterial suspensions of  $8 \times 10^5$  cfu/ml as for the HR assay, below. Plants were then covered with clear plastic bags and incubated under fluorescent lights (16 hr/day) at 22°C for 5-7 days. Leaves were scored for necrosis and chlorosis.

**Example 6 - HR assays.**

20 Tobacco leaf panels (*Nicotiana tabacum* L. 'xanthi') were infiltrated with bacterial cell suspensions as described previously (Wei, Z. M., et al., *Science*, 257:85-88 (1992); Bauer, D. W., et al., *Mol. Plant-Microbe Interact.*, 4:493-99 (1991), which are hereby incorporated by reference). Primary leaves of 2-week-old soybean seedlings (secondary leaves emerging) were infiltrated with bacterial cell suspensions as for tobacco. Plants were scored for HR (tissue collapse) after 24-48 hr on the laboratory bench. *E. amylovora* strains were suspended in 5 mM KPO<sub>4</sub> buffer, pH 6.8, and *P. syringae* strains in 10 mM MgCl<sub>2</sub>.

**Example 7 - GUS assays.**

30 Cells were 1.) grown in LB to an OD<sub>620</sub> of 0.9-1.0; 2.) grown in LB to an OD<sub>620</sub> of 0.5, then washed and resuspended in a *hrp*-gene-inducing minimal medium (Hrp MM; Huynh, T. V., et al., *Science*, 345:1374-77 (1989), which is

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hereby incorporated by reference) to an OD<sub>620</sub> of 0.2 and incubated at 21° C for 36 hr to a final OD<sub>620</sub> of 0.9-1.0; or 3.) grown in LB to an OD<sub>620</sub> of 0.5, washed and concentrated 2-fold in 5 mM KPO<sub>4</sub> buffer, pH 6.8, and then transferred to freshly cut wells in pear halves and incubated as for the pathogenicity assay for 36 hr. Cells were

5 assayed for β-glucuronidase (GUS) activity essentially according to Jefferson (Jefferson, R. A., Plant Molecular Biology Reporter, 5:387-405 (1987), which is hereby incorporated by reference). For the cells in LB or Hrp MM, 50 μl were mixed with 200 μl GUS extraction buffer (50 mM NaHPO<sub>4</sub>, pH 7.0, 10 mM β-mercaptoethanol, 10 mM Na<sub>2</sub>EDTA, 0.1% sodium lauryl sarcosine, 0.1% Triton

10 X-100) containing 2 mM 4-methylumbelliferyl β-D-glucuronide as substrate and incubated at 37° C for 100 min. For cells in pear fruit, the tissue surrounding the well was excised using a #4 cork borer and homogenized in 5 mM KPO<sub>4</sub> buffer, pH 6.8. 200 μl of homogenate was mixed with 800 μl of GUS extraction buffer with substrate and incubated as above. Reactions were stopped by adding Na<sub>2</sub>CO<sub>3</sub> to a final

15 concentration of 0.2 M in a total volume of 2 ml. Fluorescence was measured using a TKO 100 Mini-Fluorometer (Hoefer Scientific Instruments, San Francisco, CA). For all samples, cell concentration was estimated by dilution plating, and fluorometric readings were converted to pmole of substrate hydrolyzed/10<sup>8</sup> cfu/min, after Miller (Miller, J. H., A Short Course in Bacterial Genetics: A Laboratory Manual and

20 Handbook for Escherichia coli and Related Bacteria (Cold Spring Harbor Laboratory Press, Plainview, NY) (1992), which is hereby incorporated by reference).

**Example 8 - The “disease-specific” (dsp) region of *E. amylovora* consists of a 6.6 kb, two-gene operon.**

25 Mapping of previous transposon insertions (Steinberger, E. M., et al., Mol. Plant-Microbe Interact., 1:135-44 (1988), which is hereby incorporated by reference) that abolish pathogenicity but not HR-eliciting ability confirmed the presence of the “disease specific” (dsp) region downstream of the *hrpN* gene in strain Ea321 as reported in strain CFBP1430 (Barny, A. M., et al., Mol. Microbiol., 4:777-

30 86 (1990), which is hereby incorporated by reference). The sequence of approximately 15 kb of DNA downstream of *hrpN* from Ea321 was determined, revealing several open reading frames (ORFs' Fig. 1). One ORF, in an apparent 6.6 kb operon with a

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smaller ORF, spanned the area to which the *dsp* insertions mapped. These two ORFs were designated *dspE* and *dspF*, and the operon, *dspE*. *dspE* is preceded (beginning 70 bp upstream of the initiation codon) by the sequence GGAACCN<sub>15</sub>CAACATAA, which matches the HrpL-dependent promoter consensus sequence or "hrpbox" of *E. amylovora* (Kim, J. H., et al., J. Bacteriol., 179:1690-97 (1997); Kim, J. H., et al., J. Bacteriol., 179:1690-97 (1997), which are hereby incorporated by reference) and strongly resembles the *hrp* box of *P. syringae* *hrp* and *avr* genes (Xiao, Y., et al., J. Bacteriol., 176:3089-91 (1994), which is hereby incorporated by reference). Immediately downstream of *dspF* is A/T-rich DNA, followed by an ORF (ORF7) highly similar to the *Salmonella typhimurium* gene *spvR*, a member of the *lysR* family of regulatory genes (Caldwell, A. L. & Gulig, P. A., J. Bacteriol., 173:7176-85 (1991), which is hereby incorporated by reference). Immediately upstream of the *dspE* operon is a Hrp-regulated gene, *hrpW*, encoding a novel harpin.

The deduced product of *dspE* contains 1838 amino acid residues and is hydrophilic. The predicted molecular weight, 198 kD, was confirmed by expression in *E. coli* (Fig. 2). Expression of an intermediate clone containing only the 5' half of *dspE* yielded a protein of corresponding predicted mobility, suggesting that the N-terminal half of the protein might form an independently stable domain. *DspF*, predicted to be 16 kD, acidic (pI, 4.45), and predominantly  $\alpha$ -helical, with amphipathic  $\alpha$  helices in its C-terminus, is physically similar to virulence factor chaperones of animal-pathogenic bacteria (Wattiau, P., et al., Mol. Microbiol., 20:255-62 (1996), which is hereby incorporated by reference).

#### **Example 9 - *dspE* is required for fire blight.**

Two in-frame deletions within *dspE* (Fig. 1) were made in Ea321 and Ea273 (low- and high-virulence strains, respectively). The first ( $\Delta$ 1554) corresponds to amino acid residues G<sub>203</sub> to G<sub>720</sub>, and the second ( $\Delta$ 1521) to amino acid residues T<sub>1064</sub> to V<sub>1570</sub>. Each deletion abolished the ability of both strains to cause fire blight when inoculated to immature pear fruit (Fig. 3), apple shoots, or cotoneaster shoots.  $\Delta$ 1554 was complemented by a clone carrying only the overlapping 5' half of *dspE*, further suggesting that the N-terminus of the protein forms a stable domain (Figs. 1 and 3).



**Example 10 - The *dspE* operon contributes quantitatively and in a strain-dependent fashion to HR elicitation by *E. amylovora* in tobacco and is not required for HR elicitation by *E. amylovora* in soybean.**

Transposon insertions in the *dsp* region reduce the ability of *E. amylovora* to elicit the HR in tobacco (Barney, A. M., et al., *Mol. Microbiol.*, 4:777-86 (1990), which is hereby incorporated by reference). Dilution series of suspensions of *dspE*Δ1554 mutant strains of Ea321 and Ea273 were infiltrated into tobacco leaves alongside their wild-type parents to assess the role of *dspE* in HR elicitation (Fig. 3). All strains were capable of eliciting the HR, but Ea321 *dspE*Δ1554, on a per-cell basis, was roughly one-tenth as effective as the wild-type in eliciting tissue collapse. There was no noticeable difference in HR-eliciting activity, however, between Ea273 and Ea273*dspE*Δ1554. Ea321*dspE*Δ1554 elicited wild-type HR in Acme, Centennial, Harasoy, and Norchief soybean leaves (Fig. 3).

**Example 11 - The *dspE* operon is Hrp-regulated.**

A promoterless *uidA* gene construct was cloned downstream of the *dspE* fragment in pCPP1241 that was used to introduce the Δ1554 mutation (Fig. 1) into wild-type strains of *E. amylovora* (this construct consists of a 3'-truncated *dspE* gene with the internal deletion). The resulting plasmid, pCPP1263, was mobilized into Ea321 and Ea273. Pathogenic strains, in which plasmid integration had preserved an intact copy of *dspE*, and non-pathogenic strains, in which the native copy of *dspE* had been mutated, were isolated. All strains were assayed for GUS activity in Luria Bertani medium (LB) and in Hrp MM, and pathogenic strains were assayed for activity in pear fruit. High levels of activity were obtained from strains incubated in Hrp MM and pear, but not LB. The level of expression in Hrp MM was equivalent to that of a *hrcV-uidA* fusion ("G73", Wei, et al., *J. Bacteriol.*, 177:6201-10 (1995), which is hereby incorporated by reference) used as a positive control. There were no significant differences in levels of expression of the *dspE-uidA* fusion in the wild-type and *dspE* mutant backgrounds, indicating that *dspE* likely is not autoregulated. Expression of the *dspE-uidA* fusion in *hrpL* mutants of Ea321 and

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Ea273 in *hrp* MM was two orders of magnitude lower than that in HrpL + strains. Data for Ea273 and derivatives are shown in Fig. 4.

5 **Example 12 - *dspE* and *dspF* are homologous with genes in the *avrE* locus of *Pseudomonas syringae* pv. tomato.**

A BLAST (Altschul, S. F., et al., J. Mol. Biol., 215:403-10 (1990), which is hereby incorporated by reference) search of the genetic databases revealed similarity of *dspE* to a partial sequence of the *avrE* locus of *P. syringae* pv. tomato (Lorang, J. M., et al., Mol. Plant-Microbe Interact., 8:49-57 (1995), which is hereby  
10 incorporated by reference). A cosmid library of *P. syringae* pv. tomato DC3000 genomic DNA was constructed, and a clone overlapping the *hrp* gene cluster and containing the *avrE* locus was isolated (pCPP2357). The complete nucleotide sequence of the *avrE* locus was determined, revealing the homolog of *dspE* (encoding a 195 kD, 1795 amino acid protein of 30% identity) alone in an operon previously  
15 designated transcription unit III, and a homolog of *dspF* (encoding a 14 kD, a 129 amino acid protein of 43% identity) at the end of the juxtaposed and opposing operon previously designated transcription unit IV (Fig. 1). These genes are designated *avrE* and *avrF*. The C-terminal half of the DspE and AvrE alignment (from V<sub>845</sub> of DspE) shows greater conservation (33% identity) than the N-terminal half (26% identity).  
20 AvrE contains a motif (aa residues A<sub>450</sub> to T<sub>457</sub>) conserved in ATP- or GTP-binding proteins ("P-loop"; Saraste, M., et al., Trends Biochem. Sci., 15:430-34 (1990), which is hereby incorporated by reference). This motif is not conserved in DspE, however, and its functional significance in AvrE, if any, is unclear. Amino acid identities are distributed equally throughout the DspF and AvrF alignment, and AvrF shares the  
25 predicted physical characteristics of DspF. Upstream of *avrF*, completing the operon, is a 2.5 kb gene with no similarity to sequences in the genetic databases.

**Example 13 - The *dspE* operon functions as an avirulence locus.**

The *dspE* operon was cloned into pML 122 (Labes, M., et al., Gene,  
30 89:37-46 (1990), which is hereby incorporated by reference) downstream of the *npIII* promoter, and this construct, pCPP1250, was mobilized into *P. syringae* pv. glycinea race 4. The resulting strain, but not a control strain containing pML 122, elicited the HR in soybean cultivars Acme, Centennial, Harasoy, and Norchief; in Norchief plants

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incubated under conducive conditions, race 4 harboring pCPP1250 failed to cause symptoms of disease, while the control strain caused necrosis and chlorosis that spread from the point of inoculation (Fig. 5).

5 **Example 14 - *avrE* complements *dspE* mutations.**

Cosmid pCPP2357 was mobilized into Ea321 *dspE* mutant strains  $\Delta$ 1554 and  $\Delta$ 1521. The resulting transconjugants were pathogenic but low in virulence. Ea321*dspE* $\Delta$ 1521 carrying pCPP2357 with a transposon insertion in the *avrE* gene was non-pathogenic, demonstrating that the complementation observed  
10 was *avrE*-specific (Figs. 1 and 5). The same results were observed for transconjugants of the Ea273*dspE* $\Delta$ 1521 mutant.

Over thirty bacterial *avr* genes have been discovered. The plethora of *avr* genes is thought to result from an "evolutionary tug-of-war" (Dangl, J. L., in Bacterial Pathogenesis of Plants and Animals: Molecular and Cellular Mechanisms  
15 (Current Topics in Microbiology and Immunology), Dangl, J. L., ed. (Springer, Berlin), 192:99-118 (1994), which is hereby incorporated by reference), a reiterative process of selection, counterselection due to *R* genes, and modification or substitution of *avr* genes that was originally discerned by Flor, who hypothesized that "during their parallel evolution host and parasite developed complementary genic systems"  
20 (Flor, H. H., Adv. Genet., 8:29-54 (1956), which is hereby incorporated by reference). However, only a few *avr* genes (including *avrE* in strain PT23) play detectable roles in virulence or pathogen fitness in their native genetic background (Lorang, J. M., et al., Mol. Plant-Microbe Interact., 7:508-15 (1994); Kearney, B., et al., Nature, 346:385-86 (1990); Swarup, S., et al., Phytopathology, 81:802-808 (1991); De Feyter, R. D., et al., Mol. Plant-Microbe Interact., 6:225-37 (1993); Ritter, C., et al., Mol.  
25 Plant-Microbe Interact., 8:444-53 (1995), which are hereby incorporated by reference), and the selective force driving the maintenance in pathogen genomes of many of these host-range-limiting factors has remained a mystery. It is now clear, though, that several Avr proteins are delivered into plant cells by the Hrp pathway  
30 (Gopalan, S., et al., Plant Cell, 8:1095-1105 (1996); Tang, X., et al., Science, 274:2060-63 (1996); Scofield, S. R., et al., Science, 274:2063-65 (1996); Leister, R. T., et al., Proc. Natl. Acad. Sci. USA, 93:15497-15502 (1996); Van Den Ackerveken,

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G., et al., Cell, 87:1307-16 (1996), which are hereby incorporated by reference) and, therefore, are likely to be fundamentally virulence factors, which interact (directly, or indirectly through enzymatic products) with host targets to promote parasitism.

5 Mutation of such targets (selected because of reduced susceptibility) as well as the evolution of R proteins that recognize the Avr proteins would force the acquisition or evolution of new or modified Avr proteins and result in the proliferation of *avr* genes. Cumulatively, these co-evolutionary processes likely would drive a trend toward *avr* genes with quantitative and redundant effects in pathogenesis rather than critically important roles (Alfano, J. R., et al., Plant Cell, 8:1683-16988 (1996), which is hereby  
10 incorporated by reference).

It has been found that the homologs *dspE* and *avrE* contribute to disease to dramatically different extents. The avirulence locus can substitute transgenerically for the pathogenicity operon, and that the avirulence function of *dspE* extends across pathogen genera as well. These findings support the hypothesis that  
15 *avr* genes have a primary function in disease. Moreover, they support and expand the coevolutionary model for *avr* gene proliferation discussed above, and they have practical implications concerning the control of fire blight and other bacterial diseases of perennials.

One can predict from the model that the relative contribution to  
20 pathogenicity of a particular factor would reflect, in part, the genetic history of the pathogen, specifically, the degree of co-evolution with its host(s). *dspE* is required for pathogenicity; *avrE* has a quantitative, strain-dependent, virulence phenotype. Consistent with the prediction, evolution of corresponding *R* genes and modification  
of targets of pathogen virulence factors is likely to have occurred more often and to a  
25 greater extent over time in the herbaceous hosts typically infected by *P. syringae* pathogens than in the woody hosts with which *E. amylovora* presumably evolved. Alternatively or additionally, acquisition of *dspE* (through evolution or horizontal transfer) by *E. amylovora* could have occurred relatively more recently than  
acquisition of *avrE* by *P. syringae*, allowing less time for coevolution leading to  
30 modification or the development of redundant function.

One could also hypothesize from the model that virulence factors may be conserved among pathogens, yet individually adapted to avoid detection on a

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particular host. Preliminary results from Southern blot hybridizations suggest that *P. syringae* pv. *glycinea* harbors an *avrE* homolog, which, if functional, would support such a hypothesis. Similarly, homologs of the soybean cultivar-specific genes *avrA* and *avrD* from *P. syringae* pv. *tomato* exist in *P. syringae* pv. *glycinea* (Kobayashi, D. Y., et al., Proc. Natl. Acad. Sci. USA, 86:157-161 (1989), which is hereby incorporated by reference).

The homology and abilities of *dspE* and *avrE* to function transgenerically expand the model for *avr* gene proliferation. Major components of an evolution toward multifactor virulence could be procurement of genes encoding novel virulence factors from heterologous pathogens, and conservation of a functionally cosmopolitan virulence factor delivery system (and possibly conservation of a universal Hrp-pathway-targeting signal on the factors themselves) that would enable their deployment. Indeed, many *avr* genes are on plasmids and scattered in their distribution among pathogen strains (Dangl, J. L., in Bacterial Pathogenesis of Plants and Animals: Molecular and Cellular Mechanisms (Current Topics in Microbiology and Immunology), Dangl, J. L., ed. (Springer, Berlin), 192:99-118 (1994), which is hereby incorporated by reference), and individual *hrp* genes are conserved and even interchangeable (Arlat, M., et al., Mol. Plant-Microbe Interact., 4:593-601 (1991); Laby, R. J., et al., Mol. Plant-Microbe Interact., 5:412-19 (1992), which is hereby incorporated by reference). The presence of *dspE* and *avrE* in distinct genera suggests horizontal transfer of an ancestral locus, and, although *dspE* and *avrE* are homologous and *hrp*-linked, the transgeneric function of these genes suggests that the Hrp pathways in *E. amylovora* and *P. syringae* have remained insensitive to differences accrued in DspE and AvrE over evolution. It is predicted that even non-homologous Avr-like proteins will function across phytopathogenic bacterial genera.

It remains to be shown whether the avirulence function of the *dspE* locus is Hrp-pathway-dependent. This seems likely, and it will be important to determine the localization of the *dspE* and *dspF* gene products in the plant-bacterial interaction. The physical similarity of DspF (and AvrF) to chaperones required for type III secretion of virulence factors from animal-pathogenic bacteria (Wattiau, P., et al., Mol. Microbiol., 20:255-62 (1996), which is hereby incorporated by reference) is

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intriguing and novel in phytopathogenic bacteria. The requirement of these chaperones appears to be due to a role other than targeting to the secretion pathway (Woestyn, S., et al., Mol. Microbiol., 20:1261-71 (1996), which is hereby incorporated by reference): chaperones may stabilize proteins, maintain proteins in an appropriate conformation for secretion, or prevent premature polymerization or association with other proteins. Perhaps, DspF binds to DspE (and AvrF to AvrE) and plays a similar role, which might be particularly important for the latter protein due to its large size and probable multidomain nature.

The *dspE* operon is the first described avirulence locus in *E. amylovora*. A homolog of *avrRxv* from *Xanthomonas campestris* (Whalen, M. C., et al., Proc. Natl. Acad. Sci. USA, 85:6743-47 (1988), which is hereby incorporated by reference) has been found near the *dspE* operon (Kim, J. F., in Molecular Characterization of a Novel Harpin and Two hrp Secretory Operons of Erwinia amylovora, and a hrp Operon of E. chrysanthemi (Ph.D. Thesis), Cornell University, Ithaca, NY (1997)). Monogenic (*R*-gene-mediated) resistance to fire blight has not been reported, but differential virulence of *E. amylovora* strains on apple cultivars has been observed (Norelli, J. L., et al., Phytopathology, 74:136-39 (1984), which is hereby incorporated by reference). Also, some strains of *E. amylovora* infect *Rubus* spp. and not pomaceous plants, and vice-versa (Starr, M. P., et al., Phytopathology, 41:915-19 (1951), which is hereby incorporated by reference). Whether the *dspE* operon and the *avrRxv* homolog or other potential elicitors play a role in these specificities should be determined.

Although the *dspE* operon triggers defense responses in soybean when expressed in *P. syringae* pv. *glycinea*, it is not required for the HR of soybean elicited by *E. amylovora*. Neither is *hrpN* required (Fig. 3). It is possible that *E. amylovora* must have one or the other, *dspE* or *hrpN*, to elicit the HR in soybean. It has been observed, however, that purified harpin does not elicit the HR in soybean, suggesting the alternative explanation that *E. amylovora* harbors another *avr* gene recognized by this plant.

Recognition of *E. amylovora* avirulence signals in soybean indicates the presence of one or more *R* genes that might be useful for engineering fire blight resistant apple and pear trees. *R*-gene-mediated resistance to the apple scab pathogen

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*Venturia inaequalis* (Williams, E. B., et al., Ann. Rev. Phytopathol., 7:223-46 (1969), which is hereby incorporated by reference) and successful transformation of apple with attacin E for control of fire blight (Norelli, J. L., et al., Euphytica, 77:123-28 (1994), which is hereby incorporated by reference) attest the feasibility of such an approach. *R* gene-mediated resistance to apple scab has been overcome in the field (Parisi, L., et al., Phytopathology, 83:533-37 (1993), which is hereby incorporated by reference), but the requirement for *dspE* in disease favors relative durability of a *dspE*-specific *R* gene (Kearney, B. et al., Nature, 346:385-86 (1990), which is hereby incorporated by reference). Avirulence screening of *dspE* and other *E. amylovora* genes in pathogens of genetically tractable plants such as *Arabidopsis* could broaden the pool of candidate *R* genes and hasten their isolation. A similar approach could be used to isolate *R* genes effective against other diseases of woody plants. Furthermore, if the *dspE* operon is as widely conserved as is suggested by its homology with the *avrE* locus, a corresponding *R* gene could be effective against a variety of pathogens both of woody and herbaceous plants.

Native (non-denatured) DspE protein has not been produced in sufficient quantity to test its ability to elicit the HR (i.e. hypersensitive response) in a manner similar to hypersensitive response elicitors (i.e., by exogenous application). Therefore, no one has shown that *dspE* of *E. amylovora* elicits the HR when applied to plants as an isolated cell-free material. However, when the gene encoding the protein is transferred to another bacterium (along with the smaller *dspF* gene), e.g., *Pseudomonas syringae*, which ordinarily causes disease on certain plants, the recipient bacterium no longer causes disease but instead elicits the HR. The mechanism for this is not known for sure, but it is suspected to involve (and there is compelling evidence for) a mechanism in which the bacterial cell actually injects the DspE protein into the living plant cell, triggering the development of plant cell collapse (i.e. HR). Presumably, when the DspE protein is in the living plant cell, it might signal the plant to develop resistance to insects and pathogens.

Based on the similarity of the predicted physical characteristics of DspF to those of known chaperone proteins from animal pathogens, it is believed that this rather small protein is a chaperone of DspE. Chaperones in animal pathogens bind in the cytoplasm to specific proteins to be secreted. They seem to be required for

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secretion of the proteins but are not themselves secreted. Evidence suggests that the chaperones are not involved directly in targeting the secreted proteins to the secretion apparatus. Instead, they may act to stabilize the proteins in the cytoplasm and/or prevent their premature aggregation or association with other proteins (e.g., bacterial proteins that direct transport through the host cell-membrane).

The dspE gene bears no similarity to known genes except avrE. Enzymatic function (i.e., one resulting in the production of a secondary molecule that elicits the HR) of DspE cannot be ruled out at present. In fact, one avr gene product is known to elicit HR indirectly by catalyzing synthesis of a diffusible elicitor molecule. However, the simplest explanation for the observed HR eliciting function of the dspE operon expressed in *Pseudomonas* species is that the protein encoded by the dspE gene is secreted from the bacterium and possibly transported into the plant cell, that there it triggers directly plant defense responses leading to the HR, and that this process is mediated by a specific resistance gene product that recognizes (acts as a receptor of) the DspE protein. Indeed, four avr genes that depend on the Hrp secretory apparatus to function when expressed in bacteria have been shown to cause HR when expressed transgenically within plant cells. One of these has been shown to encode a protein that directly interacts with the product of its corresponding resistance gene. Ultimately, whether DspE elicits plant defense responses from outside or inside the plant cell, directly or through a secondary molecule, must be determined in order to define practical applications of this protein and its encoding gene as a plant defense elicitor.

Although the invention has been described in detail for the purpose of illustration, it is understood that such detail is solely for that purpose, and variations can be made therein by those skilled in the art without departing from the spirit and scope of the invention which is defined by the following claims.



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## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- (i) APPLICANT: Cornell Research Foundation, Inc.
- (ii) TITLE OF INVENTION: HYPERSENSITIVE RESPONSE ELICITOR FROM  
ERWINIA AMYLOVORA, ITS USE, AND  
ENCODING GENE
- (iii) NUMBER OF SEQUENCES: 5
- (iv) CORRESPONDENCE ADDRESS:
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  - (C) CITY: Rochester
  - (D) STATE: New York
  - (E) COUNTRY: U.S.A.
  - (F) ZIP: 14603
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Floppy disk
  - (B) COMPUTER: IBM PC compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
  - (A) APPLICATION NUMBER:
  - (B) FILING DATE:
  - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
  - (A) APPLICATION NUMBER: US 60/055,105
  - (B) FILING DATE: 06-AUG-1997
- (viii) ATTORNEY/AGENT INFORMATION:
  - (A) NAME: Goldman, Michael L.
  - (B) REGISTRATION NUMBER: 30,727
  - (C) REFERENCE/DOCKET NUMBER: 19603/1662
- (ix) TELECOMMUNICATION INFORMATION:
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## (2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 5517 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single

- 48 -

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATGGAATTAA AATCACTGGG AACTGAACAC AAGGCGGCAG TACACACAGC GGCGCACAAAC	60
CCTGTGGGGC ATGGTGTGTC CTTACAGCAG GGCAGCAGCA GCAGCAGCCC GCAAAATGCC	120
GCTGCATCAT TGGCGGCAGA AGGCAAAAAT CGTGGGAAAA TGCCGAGAAT TCACCAGCCA	180
TCTACTGCGG CTGATGGTAT CAGCGCTGCT CACCAGCAAA AGAAATCCTT CAGTCTCAGG	240
GGCTGTTTGG GGACGAAAAA ATTTTCCAGA TCGGCACCGC AGGGCCAGCC AGGTACCACC	300
CACAGCAAAG GGGCAACATT GCGCGATCTG CTGGCGCGGG ACGACGGCGA AACGCAGCAT	360
GAGGCGGCCG CGCCAGATGC GGCGCGTTTG ACCCGTTCGG GCGGCGTCAA ACGCCGCAAT	420
ATGGACGACA TGGCCGGGCG GCCAATGGTG AAAGGTGGCA GCGGCGAAGA TAAGGTACCA	480
ACGCAGCAAA AACGGCATCA GCTGAACAAT TTTGGCCAGA TGCGCCAAAC GATGTTGAGC	540
AAAATGGCTC ACCCGGCTTC AGCCAACGCC GGCGATCGCC TGCAGCATTG ACCGCCGCAC	600
ATCCCGGGTA GCCACCACGA AATCAAGGAA GAACCGGTTG GCTCCACCAG CAAGGCAACA	660
ACGCCCCACG CAGACAGAGT GGAAATCGCT CAGGAAGATG ACGACAGCGA ATTCCAGCAA	720
CTGCATCAAC AGCGGCTGGC GCGCGAACGG GAAAATCCAC CGCAGCCGCC CAAACTCGGC	780
GTTGCCACAC CGATTAGCGC CAGGTTTCAG CCCAAACTGA CTGCGGTTGC GGAAAGCGTC	840
CTTGAGGGGA CAGATACCAC GCAGTCACCC CTTAAGCCGC AATCAATGCT GAAAGGAAGT	900
GGAGCCGGGG TAACGCCGCT GCGGTAACG CTGGATAAAG GCAAGTTGCA GCTGGCACCG	960
GATAATCCAC CCGCGCTCAA TACGTTGTTG AAGCAGACAT TGGGTAAAGA CACCCAGCAC	1020
TATCTGGCGC ACCATGCCAG CAGCGACGGT AGCCAGCATC TGCTGCTGGA CAACAAAGGC	1080
CACCTGTTTG ATATCAAAAG CACCGCCACC AGCTATAGCG TGCTGCACAA CAGCCACCCC	1140
GGTGAGATAA AGGGCAAGCT GGCGCAGGCG GGTACTGGCT CCGTCAGCGT AGACGGTAAA	1200
AGCGGCAAGA TCTCGCTGGG GAGCGGTACG CAAAGTCACA ACAAACAAT GCTAAGCCAA	1260
CCGGGGGAAG CGCACCGTTC CTTATTAACC GGCATTTGGC AGCATCCTGC TGGCGCAGCG	1320
CGGCCGCAGG GCGAGTCAAT CCGCCTGCAT GACGACAAAA TTCATATCCT GCATCCGGAG	1380
CTGGGCGTAT GGCAATCTGC GGATAAAGAT ACCCAGAGCC AGCTGTCTCG CCAGGCAGAC	1440
GGTAAGCTCT ATGCGCTGAA AGACAACCGT ACCCTGCAAA ACCTCTCCGA TAATAAATCC	1500
TCAGAAAAGC TGGTCGATAA AATCAAATCG TATTCCGTTG ATCAGCGGGG GCAGGTGGCG	1560
ATCCTGACGG ATACTCCCGG CCGCCATAAG ATGAGTATTA TGCCCTCGCT GGATGCTTCC	1620

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CCGGAGAGCC ATATTTCCCT CAGCCTGCAT TTTGCCGATG CCCACCAGGG GTTATTGCAC	1680
GGGAAGTCGG AGCTTGAGGC ACAATCTGTC GCGATCAGCC ATGGGCGACT GGTGTGGCC	1740
GATAGCGAAG GCAAGCTGTT TAGCGCCGCC ATTCCGAAGC AAGGGGATGG AAACGAACTG	1800
AAAATGAAAG CCATGCCTCA GCATGCGCTC GATGAACATT TTGGTCATGA CCACCAGATT	1860
TCTGGATTTT TCCATGACGA CCACGGCCAG CTTAATGCGC TGGTGAAAAA TAACTTCAGG	1920
CAGCAGCATG CCTGCCCGTT GGGTAACGAT CATCAGTTTC ACCCGGCTG GAACCTGACT	1980
GATGCGCTGG TTATCGACAA TCAGCTGGGG CTGCATCATA CCAATCCTGA ACCGCATGAG	2040
ATTCTTGATA TGGGGCATT AGGCAGCTG GCGTTACAGG AGGGCAAGCT TCACTATTTT	2100
GACCAGCTGA CCAAAGGGTG GACTGGCGCG GAGTCAGATT GTAAGCAGCT GAAAAAAGGC	2160
CTGGATGGAG CAGCTTATCT ACTGAAAGAC GGTGAAGTGA AACGCCTGAA TATTAATCAG	2220
AGCACCTCCT CTATCAAGCA CGGAACGGAA AACGTTTTTT CGCTGCCGCA TGTGCGCAAT	2280
AAACCGGAGC CGGGAGATGC CTGCAAGGG CTGAATAAAG ACGATAAGGC CCAGGCCATG	2340
GCGGTGATTG GGGTAAATAA ATACCTGGCG CTGACGGAAA AAGGGGACAT TCGTCTCTTC	2400
CAGATAAAAC CCGGCACCCA GCAGTTGGAG CGGCCGGCAC AAACCTCAG CCGCGAAGGT	2460
ATCAGCGCG AACTGAAAGA CATTTCATGTC GACCACAAGC AGAACCTGTA TGCCTTGACC	2520
CACGAGGGAG AGGTGTTTCA TCAGCCGCGT GAAGCCTGGC AGAATGGTGC CGAAAGCAGC	2580
AGCTGGCACA AACTGGCGTT GCCACAGAGT GAAAGTAAGC TAAAAAGTCT GGACATGAGC	2640
CATGAGCACA AACCGATTGC CACCTTTGAA GACGGTAGCC AGCATCAGCT GAAGGCTGGC	2700
GGCTGGCAGC CCTATGCGGC ACCTGAACGC GGGCCGCTGG CGGTGGGTAC CAGCGTTCA	2760
CAAACCGTCT TTAACCGACT AATGCAGGG GTGAAAGGCA AGGTGATCCC AGGCAGCGGG	2820
TTGACGGTTA AGCTCTCGGC TCAGACGGGG GGAATGACCG GCGCCGAAGG GCGCAAGGTC	2880
AGCAGTAAAT TTTCCGAAAG GATCCGCGCC TATGCGTTCA ACCCAACAAT GTCCACGCCG	2940
CGACCGATTA AAAATGCTGC TTATGCCACA CAGCACGGCT GGCAGGGGCG TGAGGGGTTG	3000
AAGCCGTTGT ACGAGATGCA GGGAGCGCTG ATTAAACAAC TGGATGCGCA TAACGTTCTG	3060
CATAACGCGC CACAGCCAGA TTTGCAGAGC AAACCTGAAA CTCTGGATTT AGGCGAACAT	3120
GGCGCAGAAT TGCTTAACGA CATGAAGCGC TTCCGCGACG AACTGGAGCA GAGTGCAACC	3180
CGTTCGGTGA CCGTTTTAGG TCAACATCAG GGAGTGCTAA AAAGCAACGG TGAAATCAAT	3240
AGCGAATTTA AGCCATCGCC CGGCAAGGCG TTGGTCCAGA GCTTTAACGT CAATCGCTCT	3300
GGTCAGGATC TAAGCAAGTC ACTGCAACAG GCAGTACATG CCACGCCGCC ATCCGAGAG	3360
AGTAACTGC AATCCATGCT GGGGCACTTT GTCAGTGCCG GGGTGGATAT GAGTCATCAG	3420
AAGGGCGAGA TCCCGCTGGG CCGCCAGCGC GATCCGAATG ATAAACCGC ACTGACCAA	3480

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TCGCGTTTAA TTTTAGATAC CGTGACCATC GGTGAACTGC ATGAACTGGC CGATAAGGCG	3540
AAACTGGTAT CTGACCATAA ACCCGATGCC GATCAGATAA AACAGCTGCG CCAGCAGTTC	3600
GATACGCTGC GTGAAAAGCG GTATGAGAGC AATCCGGTGA AGCATTACAC CGATATGGGC	3660
TTCACCCATA ATAAGGCGCT GGAAGCAAAC TATGATGCGG TCAAAGCCTT TATCAATGCC	3720
TTTAAGAAAG AGCACCACGG CGTCAATCTG ACCACGCGTA CCGTACTGGA ATCACAGGGC	3780
AGTGCGGAGC TGGCGAAGAA GCTCAAGAAT ACGCTGTTGT CCCTGGACAG TGGTGAAAGT	3840
ATGAGCTTCA GCCGGTCATA TGGCGGGGGC GTCAGCACTG TCTTTGTGCC TACCCTTAGC	3900
AAGAAGGTGC CAGTTCGGGT GATCCCCGGA GCCGGCATCA CGCTGGATCG CGCCTATAAC	3960
CTGAGCTTCA GTCGTACCAG CGGCGGATTG AACGTCAGTT TTGGCCCGCA CGGCGGGGTG	4020
AGTGGTAACA TCATGGTCGC TACCGGCCAT GATGTGATGC CCTATATGAC CGGTAAGAAA	4080
ACCACTGCAG GTAACGCCAG TGACTGGTTG AGCGCAAAAC ATAAATCAG CCCGGAATTG	4140
CGTATCGGCG CTGCTGTGAG TGGCACCTG CAAGGAACGC TACAAAACAG CCTGAAGTTT	4200
AAGCTGACAG AGGATGAGCT GCCTGGCTTT ATCCATGGCT TGACGCATGG CACGTTGACC	4260
CCGGCAGAAC TGTGCAAAA GGGGATCGAA CATCAGATGA AGCAGGGCAG CAACTGACG	4320
TTAGCGTCG ATACCTCGGC AAATCTGGAT CTGCGTGCCG GTATCAATCT GAACGAAGAC	4380
GGCAGTAAAC CAAATGGTGT CACTGCCCCG GTTCTGCGG GGCTAAGTGC ATCGGCAAAC	4440
CTGGCCGCCG GCTCGCGTGA ACGCAGCACC ACCTCTGGCC AGTTTGGCAG CACGACTTCG	4500
GCCAGCAATA ACCGCCCAAC CTTCTCAAC GGGGTCGGCG CGGGTGCTAA CCTGACGGCT	4560
GCTTTAGGGG TTGCCCATTG ATCTACGCAT GAAGGGAAC CGGTCGGGAT CTTCCCGGCA	4620
TTTACCTCGA CCAATGTTTC GGCAGCGCTG GCGCTGGATA ACCGTACCTC ACAGAGTATC	4680
AGCCTGGAAT TGAAGCGCGC GGAGCCGGTG ACCAGCAACG ATATCAGCGA GTTGACCTCC	4740
ACGCTGGGAA AACACTTTAA GGATAGCGCC ACAACGAAGA TGCTTGCCGC TCTCAAAGAG	4800
TTAGATGACG CTAAGCCGCG TGAACAACCTG CATATTTTAC AGCAGCATTT CAGTGCAAAA	4860
GATGTCGTCG GTGATGAACG CTACGAGGCG GTGCGCAACC TGAAAAAACT GGTGATACGT	4920
CAACAGGCTG CGGACAGCCA CAGCATGGAA TTAGGATCTG CCAGTCACAG CACGACCTAC	4980
AATAATCTGT CGAGAATAAA TAATGACGGC ATTGTCGAGC TGCTACACAA ACATTTTCGAT	5040
GCGGCATTAC CAGCAAGCAG TGCCAAACGT CTTGGTGAAA TGATGAATAA CGATCCGGCA	5100
CTGAAAGATA TTATTAAGCA GCTGCAAAGT ACGCCGTTCG GCAGCGCCAG CGTGTCGATG	5160
GAGCTGAAAG ATGGTCTGCG TGAGCAGACG GAAAAAGCAA TACTGGACGG TAAGGTCGGT	5220
CGTGAAGAAG TGGGAGTACT TTTCCAGGAT CGTAACAACT TGCGTGTTAA ATCGGTCAGC	5280
GTCACTCAGT CCGTCAGCAA AAGCGAAGGC TTCAATACCC CAGCGCTGTT ACTGGGGACG	5340

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AGCAACAGCG CTGCTATGAG CATGGAGCGC AACATCGGAA CCATTAATTT TAAATACGGC 5400  
 CAGGATCAGA ACACCCACG GCGATTTACC CTGGAGGGTG GAATAGCTCA GGCTAATCCG 5460  
 CAGGTCGCAT CTGCGCTTAC TGATTTGAAG AAGGAAGGGC TGGAAATGAA GAGCTAA 5517

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1838 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Glu Leu Lys Ser Leu Gly Thr Glu His Lys Ala Ala Val His Thr  
 1 5 10 15  
 Ala Ala His Asn Pro Val Gly His Gly Val Ala Leu Gln Gln Gly Ser  
 20 25 30  
 Ser Ser Ser Ser Pro Gln Asn Ala Ala Ala Ser Leu Ala Ala Glu Gly  
 35 40 45  
 Lys Asn Arg Gly Lys Met Pro Arg Ile His Gln Pro Ser Thr Ala Ala  
 50 55 60  
 Asp Gly Ile Ser Ala Ala His Gln Gln Lys Lys Ser Phe Ser Leu Arg  
 65 70 75 80  
 Gly Cys Leu Gly Thr Lys Lys Phe Ser Arg Ser Ala Pro Gln Gly Gln  
 85 90 95  
 Pro Gly Thr Thr His Ser Lys Gly Ala Thr Leu Arg Asp Leu Leu Ala  
 100 105 110  
 Arg Asp Asp Gly Glu Thr Gln His Glu Ala Ala Ala Pro Asp Ala Ala  
 115 120 125  
 Arg Leu Thr Arg Ser Gly Gly Val Lys Arg Arg Asn Met Asp Asp Met  
 130 135 140  
 Ala Gly Arg Pro Met Val Lys Gly Gly Ser Gly Glu Asp Lys Val Pro  
 145 150 155 160  
 Thr Gln Gln Lys Arg His Gln Leu Asn Asn Phe Gly Gln Met Arg Gln  
 165 170 175  
 Thr Met Leu Ser Lys Met Ala His Pro Ala Ser Ala Asn Ala Gly Asp  
 180 185 190  
 Arg Leu Gln His Ser Pro Pro His Ile Pro Gly Ser His His Glu Ile  
 195 200 205

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Lys Glu Glu Pro Val Gly Ser Thr Ser Lys Ala Thr Thr Ala His Ala  
 210 215 220  
 Asp Arg Val Glu Ile Ala Gln Glu Asp Asp Asp Ser Glu Phe Gln Gln  
 225 230 235 240  
 Leu His Gln Gln Arg Leu Ala Arg Glu Arg Glu Asn Pro Pro Gln Pro  
 245 250 255  
 Pro Lys Leu Gly Val Ala Thr Pro Ile Ser Ala Arg Phe Gln Pro Lys  
 260 265 270  
 Leu Thr Ala Val Ala Glu Ser Val Leu Glu Gly Thr Asp Thr Thr Gln  
 275 280 285  
 Ser Pro Leu Lys Pro Gln Ser Met Leu Lys Gly Ser Gly Ala Gly Val  
 290 295 300  
 Thr Pro Leu Ala Val Thr Leu Asp Lys Gly Lys Leu Gln Leu Ala Pro  
 305 310 315 320  
 Asp Asn Pro Pro Ala Leu Asn Thr Leu Leu Lys Gln Thr Leu Gly Lys  
 325 330 335  
 Asp Thr Gln His Tyr Leu Ala His His Ala Ser Ser Asp Gly Ser Gln  
 340 345 350  
 His Leu Leu Leu Asp Asn Lys Gly His Leu Phe Asp Ile Lys Ser Thr  
 355 360 365  
 Ala Thr Ser Tyr Ser Val Leu His Asn Ser His Pro Gly Glu Ile Lys  
 370 375 380  
 Gly Lys Leu Ala Gln Ala Gly Thr Gly Ser Val Ser Val Asp Gly Lys  
 385 390 395 400  
 Ser Gly Lys Ile Ser Leu Gly Ser Gly Thr Gln Ser His Asn Lys Thr  
 405 410 415  
 Met Leu Ser Gln Pro Gly Glu Ala His Arg Ser Leu Leu Thr Gly Ile  
 420 425 430  
 Trp Gln His Pro Ala Gly Ala Ala Arg Pro Gln Gly Glu Ser Ile Arg  
 435 440 445  
 Leu His Asp Asp Lys Ile His Ile Leu His Pro Glu Leu Gly Val Trp  
 450 455 460  
 Gln Ser Ala Asp Lys Asp Thr His Ser Gln Leu Ser Arg Gln Ala Asp  
 465 470 475 480  
 Gly Lys Leu Tyr Ala Leu Lys Asp Asn Arg Thr Leu Gln Asn Leu Ser  
 485 490 495  
 Asp Asn Lys Ser Ser Glu Lys Leu Val Asp Lys Ile Lys Ser Tyr Ser  
 500 505 510  
 Val Asp Gln Arg Gly Gln Val Ala Ile Leu Thr Asp Thr Pro Gly Arg  
 515 520 525  
 His Lys Met Ser Ile Met Pro Ser Leu Asp Ala Ser Pro Glu Ser His  
 530 535 540

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Ile Ser Leu Ser Leu His Phe Ala Asp Ala His Gln Gly Leu Leu His  
 545 550 555 560  
 Gly Lys Ser Glu Leu Glu Ala Gln Ser Val Ala Ile Ser His Gly Arg  
 565 570 575  
 Leu Val Val Ala Asp Ser Glu Gly Lys Leu Phe Ser Ala Ala Ile Pro  
 580 585 590  
 Lys Gln Gly Asp Gly Asn Glu Leu Lys Met Lys Ala Met Pro Gln His  
 595 600 605  
 Ala Leu Asp Glu His Phe Gly His Asp His Gln Ile Ser Gly Phe Phe  
 610 615 620  
 His Asp Asp His Gly Gln Leu Asn Ala Leu Val Lys Asn Asn Phe Arg  
 625 630 635 640  
 Gln Gln His Ala Cys Pro Leu Gly Asn Asp His Gln Phe His Pro Gly  
 645 650 655  
 Trp Asn Leu Thr Asp Ala Leu Val Ile Asp Asn Gln Leu Gly Leu His  
 660 665 670  
 His Thr Asn Pro Glu Pro His Glu Ile Leu Asp Met Gly His Leu Gly  
 675 680 685  
 Ser Leu Ala Leu Gln Glu Gly Lys Leu His Tyr Phe Asp Gln Leu Thr  
 690 695 700  
 Lys Gly Trp Thr Gly Ala Glu Ser Asp Cys Lys Gln Leu Lys Lys Gly  
 705 710 715 720  
 Leu Asp Gly Ala Ala Tyr Leu Leu Lys Asp Gly Glu Val Lys Arg Leu  
 725 730 735  
 Asn Ile Asn Gln Ser Thr Ser Ser Ile Lys His Gly Thr Glu Asn Val  
 740 745 750  
 Phe Ser Leu Pro His Val Arg Asn Lys Pro Glu Pro Gly Asp Ala Leu  
 755 760 765  
 Gln Gly Leu Asn Lys Asp Asp Lys Ala Gln Ala Met Ala Val Ile Gly  
 770 775 780  
 Val Asn Lys Tyr Leu Ala Leu Thr Glu Lys Gly Asp Ile Arg Ser Phe  
 785 790 795 800  
 Gln Ile Lys Pro Gly Thr Gln Gln Leu Glu Arg Pro Ala Gln Thr Leu  
 805 810 815  
 Ser Arg Glu Gly Ile Ser Gly Glu Leu Lys Asp Ile His Val Asp His  
 820 825 830  
 Lys Gln Asn Leu Tyr Ala Leu Thr His Glu Gly Glu Val Phe His Gln  
 835 840 845  
 Pro Arg Glu Ala Trp Gln Asn Gly Ala Glu Ser Ser Ser Trp His Lys  
 850 855 860

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Leu Ala Leu Pro Gln Ser Glu Ser Lys Leu Lys Ser Leu Asp Met Ser  
 865 870 875 880  
 His Glu His Lys Pro Ile Ala Thr Phe Glu Asp Gly Ser Gln His Gln  
 885 890 895  
 Leu Lys Ala Gly Gly Trp His Ala Tyr Ala Ala Pro Glu Arg Gly Pro  
 900 905 910  
 Leu Ala Val Gly Thr Ser Gly Ser Gln Thr Val Phe Asn Arg Leu Met  
 915 920 925  
 Gln Gly Val Lys Gly Lys Val Ile Pro Gly Ser Gly Leu Thr Val Lys  
 930 935 940  
 Leu Ser Ala Gln Thr Gly Gly Met Thr Gly Ala Glu Gly Arg Lys Val  
 945 950 955 960  
 Ser Ser Lys Phe Ser Glu Arg Ile Arg Ala Tyr Ala Phe Asn Pro Thr  
 965 970 975  
 Met Ser Thr Pro Arg Pro Ile Lys Asn Ala Ala Tyr Ala Thr Gln His  
 980 985 990  
 Gly Trp Gln Gly Arg Glu Gly Leu Lys Pro Leu Tyr Glu Met Gln Gly  
 995 1000 1005  
 Ala Leu Ile Lys Gln Leu Asp Ala His Asn Val Arg His Asn Ala Pro  
 1010 1015 1020  
 Gln Pro Asp Leu Gln Ser Lys Leu Glu Thr Leu Asp Leu Gly Glu His  
 1025 1030 1035 1040  
 Gly Ala Glu Leu Leu Asn Asp Met Lys Arg Phe Arg Asp Glu Leu Glu  
 1045 1050 1055  
 Gln Ser Ala Thr Arg Ser Val Thr Val Leu Gly Gln His Gln Gly Val  
 1060 1065 1070  
 Leu Lys Ser Asn Gly Glu Ile Asn Ser Glu Phe Lys Pro Ser Pro Gly  
 1075 1080 1085  
 Lys Ala Leu Val Gln Ser Phe Asn Val Asn Arg Ser Gly Gln Asp Leu  
 1090 1095 1100  
 Ser Lys Ser Leu Gln Gln Ala Val His Ala Thr Pro Pro Ser Ala Glu  
 1105 1110 1115 1120  
 Ser Lys Leu Gln Ser Met Leu Gly His Phe Val Ser Ala Gly Val Asp  
 1125 1130 1135  
 Met Ser His Gln Lys Gly Glu Ile Pro Leu Gly Arg Gln Arg Asp Pro  
 1140 1145 1150  
 Asn Asp Lys Thr Ala Leu Thr Lys Ser Arg Leu Ile Leu Asp Thr Val  
 1155 1160 1165  
 Thr Ile Gly Glu Leu His Glu Leu Ala Asp Lys Ala Lys Leu Val Ser  
 1170 1175 1180  
 Asp His Lys Pro Asp Ala Asp Gln Ile Lys Gln Leu Arg Gln Gln Phe  
 1185 1190 1195 1200



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Asp Thr Leu Arg Glu Lys Arg Tyr Glu Ser Asn Pro Val Lys His Tyr  
 1205 1210 1215  
 Thr Asp Met Gly Phe Thr His Asn Lys Ala Leu Glu Ala Asn Tyr Asp  
 1220 1225 1230  
 Ala Val Lys Ala Phe Ile Asn Ala Phe Lys Lys Glu His His Gly Val  
 1235 1240 1245  
 Asn Leu Thr Thr Arg Thr Val Leu Glu Ser Gln Gly Ser Ala Glu Leu  
 1250 1255 1260  
 Ala Lys Lys Leu Lys Asn Thr Leu Leu Ser Leu Asp Ser Gly Glu Ser  
 1265 1270 1275 1280  
 Met Ser Phe Ser Arg Ser Tyr Gly Gly Gly Val Ser Thr Val Phe Val  
 1285 1290 1295  
 Pro Thr Leu Ser Lys Lys Val Pro Val Pro Val Ile Pro Gly Ala Gly  
 1300 1305 1310  
 Ile Thr Leu Asp Arg Ala Tyr Asn Leu Ser Phe Ser Arg Thr Ser Gly  
 1315 1320 1325  
 Gly Leu Asn Val Ser Phe Gly Arg Asp Gly Gly Val Ser Gly Asn Ile  
 1330 1335 1340  
 Met Val Ala Thr Gly His Asp Val Met Pro Tyr Met Thr Gly Lys Lys  
 1345 1350 1355 1360  
 Thr Ser Ala Gly Asn Ala Ser Asp Trp Leu Ser Ala Lys His Lys Ile  
 1365 1370 1375  
 Ser Pro Asp Leu Arg Ile Gly Ala Ala Val Ser Gly Thr Leu Gln Gly  
 1380 1385 1390  
 Thr Leu Gln Asn Ser Leu Lys Phe Lys Leu Thr Glu Asp Glu Leu Pro  
 1395 1400 1405  
 Gly Phe Ile His Gly Leu Thr His Gly Thr Leu Thr Pro Ala Glu Leu  
 1410 1415 1420  
 Leu Gln Lys Gly Ile Glu His Gln Met Lys Gln Gly Ser Lys Leu Thr  
 1425 1430 1435 1440  
 Phe Ser Val Asp Thr Ser Ala Asn Leu Asp Leu Arg Ala Gly Ile Asn  
 1445 1450 1455  
 Leu Asn Glu Asp Gly Ser Lys Pro Asn Gly Val Thr Ala Arg Val Ser  
 1460 1465 1470  
 Ala Gly Leu Ser Ala Ser Ala Asn Leu Ala Ala Gly Ser Arg Glu Arg  
 1475 1480 1485  
 Ser Thr Thr Ser Gly Gln Phe Gly Ser Thr Thr Ser Ala Ser Asn Asn  
 1490 1495 1500  
 Arg Pro Thr Phe Leu Asn Gly Val Gly Ala Gly Ala Asn Leu Thr Ala  
 1505 1510 1515 1520

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Ala Leu Gly Val Ala His Ser Ser Thr His Glu Gly Lys Pro Val Gly  
 1525 1530 1535  
 Ile Phe Pro Ala Phe Thr Ser Thr Asn Val Ser Ala Ala Leu Ala Leu  
 1540 1545 1550  
 Asp Asn Arg Thr Ser Gln Ser Ile Ser Leu Glu Leu Lys Arg Ala Glu  
 1555 1560 1565  
 Pro Val Thr Ser Asn Asp Ile Ser Glu Leu Thr Ser Thr Leu Gly Lys  
 1570 1575 1580  
 His Phe Lys Asp Ser Ala Thr Thr Lys Met Leu Ala Ala Leu Lys Glu  
 1585 1590 1595 1600  
 Leu Asp Asp Ala Lys Pro Ala Glu Gln Leu His Ile Leu Gln Gln His  
 1605 1610 1615  
 Phe Ser Ala Lys Asp Val Val Gly Asp Glu Arg Tyr Glu Ala Val Arg  
 1620 1625 1630  
 Asn Leu Lys Lys Leu Val Ile Arg Gln Gln Ala Ala Asp Ser His Ser  
 1635 1640 1645  
 Met Glu Leu Gly Ser Ala Ser His Ser Thr Thr Tyr Asn Asn Leu Ser  
 1650 1655 1660  
 Arg Ile Asn Asn Asp Gly Ile Val Glu Leu Leu His Lys His Phe Asp  
 1665 1670 1675 1680  
 Ala Ala Leu Pro Ala Ser Ser Ala Lys Arg Leu Gly Glu Met Met Asn  
 1685 1690 1695  
 Asn Asp Pro Ala Leu Lys Asp Ile Ile Lys Gln Leu Gln Ser Thr Pro  
 1700 1705 1710  
 Phe Ser Ser Ala Ser Val Ser Met Glu Leu Lys Asp Gly Leu Arg Glu  
 1715 1720 1725  
 Gln Thr Glu Lys Ala Ile Leu Asp Gly Lys Val Gly Arg Glu Glu Val  
 1730 1735 1740  
 Gly Val Leu Phe Gln Asp Arg Asn Asn Leu Arg Val Lys Ser Val Ser  
 1745 1750 1755 1760  
 Val Ser Gln Ser Val Ser Lys Ser Glu Gly Phe Asn Thr Pro Ala Leu  
 1765 1770 1775  
 Leu Leu Gly Thr Ser Asn Ser Ala Ala Met Ser Met Glu Arg Asn Ile  
 1780 1785 1790  
 Gly Thr Ile Asn Phe Lys Tyr Gly Gln Asp Gln Asn Thr Pro Arg Arg  
 1795 1800 1805  
 Phe Thr Leu Glu Gly Gly Ile Ala Gln Ala Asn Pro Gln Val Ala Ser  
 1810 1815 1820  
 Ala Leu Thr Asp Leu Lys Lys Glu Gly Leu Glu Met Lys Ser  
 1825 1830 1835

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## (2) INFORMATION FOR SEQ ID NO:3:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 420 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```

ATGACATCGT CACAGCAGCG GGTGAAAGG TTTTACAGT ATTCTCCGC CGGGTGTA   60
ACGCCCATAC ATCTGAAAGA CGGGGTGTC GCCCTGTATA ACGAACAAGA TGAGGAGGCG   120
GCGGTGCTGG AAGTACCGCA ACACAGCGAC AGCCTGTTAC TACACTGCCG AATCATTGAG   180
GCTGACCCAC AAACCTCAAT AACCTGTAT TCGATGCTAT TACAGCTGAA TTTGAAATG   240
GCGGCCATGC GCGGCTGTTG GCTGGCGCTG GATGAACTGC ACAACGTGCG TTTATGTTTT   300
CAGCAGTCGC TGGAGCATCT GGATGAAGCA AGTTTTAGCG ATATCGTTAG CGGCTTCATC   360
GAACATGCGG CAGAAGTGCG TGAGTATATA GCGCAATTAG ACGAGAGTAG CGCGGCATAA   420

```

## (2) INFORMATION FOR SEQ ID NO:4:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 139 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```

Met Thr Ser Ser Gln Gln Arg Val Glu Arg Phe Leu Gln Tyr Phe Ser
1             5             10             15

Ala Gly Cys Lys Thr Pro Ile His Leu Lys Asp Gly Val Cys Ala Leu
20             25             30

Tyr Asn Glu Gln Asp Glu Glu Ala Ala Val Leu Glu Val Pro Gln His
35             40             45

Ser Asp Ser Leu Leu Leu His Cys Arg Ile Ile Glu Ala Asp Pro Gln
50             55             60

Thr Ser Ile Thr Leu Tyr Ser Met Leu Leu Gln Leu Asn Phe Glu Met
65             70             75             80

Ala Ala Met Arg Gly Cys Trp Leu Ala Leu Asp Glu Leu His Asn Val
85             90             95

```

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Arg Leu Cys Phe Gln Gln Ser Leu Glu His Leu Asp Glu Ala Ser Phe  
100 105 110

Ser Asp Ile Val Ser Gly Phe Ile Glu His Ala Ala Glu Val Arg Glu  
115 120 125

Tyr Ile Ala Gln Leu Asp Glu Ser Ser Ala Ala  
130 135

## (2) INFORMATION FOR SEQ ID NO:5:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GGAACCNNNN NNNNNNNNNN NCAACATAA

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**WHAT IS CLAIMED:**

1. An isolated DNA molecule encoding a hypersensitive response eliciting protein or polypeptide, wherein the isolated DNA molecule is selected from the group consisting of (a) a DNA molecule comprising a nucleotide sequence of SEQ. ID. Nos. 1 or 3, (b) a DNA molecule encoding a protein comprising an amino acid of SEQ. ID. Nos. 2 or 4, (c) a DNA molecule which hybridizes to a DNA molecule comprising a nucleotide sequence of SEQ. ID. Nos. 1 or 3, under stringent conditions, and (d) a DNA molecule complementary to DNA molecules (a), (b), and (c).
2. An isolated DNA molecule according to claim 1, wherein said DNA molecule is a DNA molecule comprising a nucleotide sequence of SEQ. ID. Nos. 1 or 3.
3. An isolated DNA molecule according to claim 1, wherein said DNA molecule is a DNA molecule encoding protein comprising an amino acid of SEQ. ID. Nos. 2 or 4.
4. An isolated DNA molecule according to claim 1, wherein said DNA molecule is a DNA molecule which hybridizes to a DNA molecule comprising a nucleotide sequence of SEQ. ID. Nos. 1 or 3, under stringent conditions.
5. An isolated DNA molecule according to claim 1, wherein said DNA molecule is a DNA molecule complementary to DNA molecules (a), (b), and (c).
6. An expression vector transformed with the DNA molecule of claim 1.
7. An expression vector according to claim 6, wherein the DNA molecule is in proper sense orientation and correct reading frame.
8. A host cell transformed with the DNA molecule of claim 1.

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9. A host cell according to claim 8, wherein the host cell is selected from the group consisting of a plant cell or a bacterial cell.

10. A host cell according to claim 8, wherein the DNA molecule is transformed with an expression vector.

11. A transgenic plant transformed with the DNA molecule of claim 1.

12. A transgenic plant according to claim 11, wherein the plant is selected from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

13. A transgenic plant according to claim 11, wherein the plant is selected from the group consisting of *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

14. A transgenic plant seed transformed with the DNA molecule of claim 1.

15. A transgenic plant seed according to claim 14, wherein the plant seed is selected from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

16. A transgenic plant seed according to claim 14, wherein the plant seed is selected from the group consisting of *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

17. An isolated hypersensitive response eliciting protein or polypeptide selected from the group consisting of a protein or polypeptide having an amino acid comprising SEQ. ID. Nos. 2 or 4, and an amino acid encoded by a nucleic acid which hybridizes to a DNA molecule comprising a nucleotide sequence of SEQ. ID. Nos. 1 or 3.

18. An isolated protein or polypeptide according to claim 17, wherein the protein or polypeptide has an amino acid comprising SEQ. ID. Nos. 2 or 4.

19. An isolated protein or polypeptide according to claim 17, wherein the protein or polypeptide is encoded by a nucleic acid which hybridizes to a DNA molecule comprising a nucleotide sequence of SEQ. ID. Nos. 1 or 3.

20. A method of imparting disease resistance to plants comprising: applying a protein or polypeptide according claim 17 in a non-infectious form to a plant or plant seed under conditions effective to impart disease resistance.

21. A method according to claim 20, wherein plants are treated during said applying.

22. A method according to claim 20, wherein plant seeds are treated during said applying, said method further comprising: planting the seeds treated with the hypersensitive response elicitor in natural or artificial soil and propagating plants from the seeds planted in the soil.

23. A method of enhancing plant growth comprising: applying a protein or polypeptide according claim 17 in a non-infectious form to a plant or plant seed under conditions effective to enhance plant growth.

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24. A method according to claim 23, wherein plants are treated during said applying.

25. A method according to claim 23, wherein plant seeds are  
5 treated during said applying, said method further comprising:  
planting the seeds treated with the hypersensitive response  
elicitor in natural or artificial soil and  
propagating plants from the seeds planted in the soil.

10 26. A method of insect control for plants comprising:  
applying a protein or polypeptide according claim 17 in a non-  
infectious form to a plant or plant seed under conditions effective to control insects.

15 27. A method according to claim 26, wherein plants are treated  
during said applying.

28. A method according to claim 26, wherein plant seeds are  
treated during said applying, said method further comprising:  
planting the seeds treated with the hypersensitive response  
20 elicitor in natural or artificial soil and  
propagating plants from the seeds planted in the soil.

29. A method of imparting disease resistance to plants comprising:  
providing a transgenic plant or plant seed transformed with a  
25 DNA molecule according to claim 1 and  
growing the transgenic plant or transgenic plants produced  
from the transgenic plant seeds under conditions effective to impart disease resistance.

30. A method according to claim 29, wherein a transgenic plant is  
30 provided.

31. A method according to claim 29, wherein a transgenic plant  
seed is provided.



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32. A method of enhancing plant growth comprising:  
providing a transgenic plant or plant seed transformed with a  
DNA molecule according to claim 1 and  
growing the transgenic plant or transgenic plants produced  
5 from the transgenic plant seeds under conditions effective to enhance plant growth.
33. A method according to claim 32, wherein a transgenic plant is  
provided.
- 10 34. A method according to claim 32, wherein a transgenic plant  
seed is provided.
35. A method of insect control for plants comprising:  
providing a transgenic plant or plant seed transformed with a  
15 DNA molecule according to claim 1 and  
growing the transgenic plant or transgenic plants produced  
from the transgenic plant seeds under conditions effective to control insects.
36. A method according to claim 35, wherein a transgenic plant is  
20 provided.
37. A method according to claim 35, wherein a transgenic plant  
seed is provided.
- 25 38. A composition comprising:  
a protein or polypeptide according to claim 17 and  
a carrier.
39. A composition according to claim 38 further comprising an  
30 additive selected from the group consisting of fertilizer, insecticide, fungicide,  
nematocide, and mixtures thereof.
40. An antibody or binding portion thereof which recognizes a protein  
or polypeptide according to claim 17.

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41. An antibody or binding portion thereof according to claim 40,  
wherein the antibody is a monoclonal antibody.

42. An antibody or binding portion thereof according to claim 40,  
5 wherein the antibody is a polyclonal antibody.

43. A method to alter the disease or hypersensitive response in a  
plant comprising:  
providing the plant with an antibody or binding portion thereof  
10 according to claim 40 and  
causing the antibody or binding portion thereof to bind to a  
hypersensitive response elicitor protein or polypeptide under conditions effective to  
alter disease or hypersensitive response.

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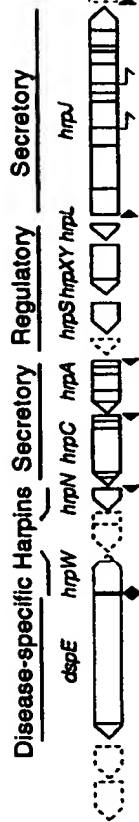


FIG. 1A

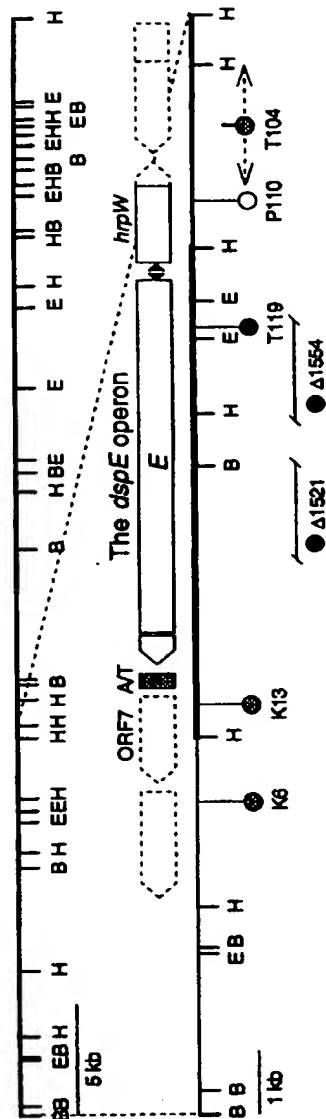


FIG. 1B

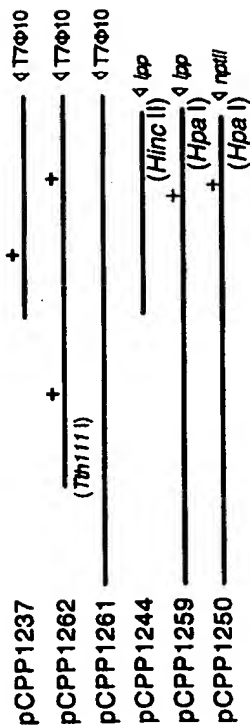


FIG. 1C

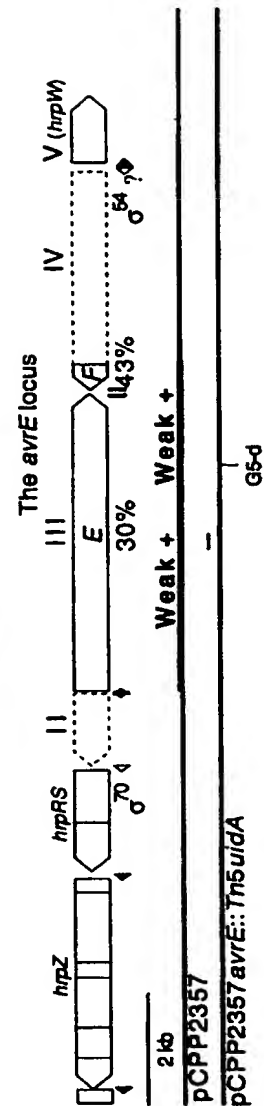
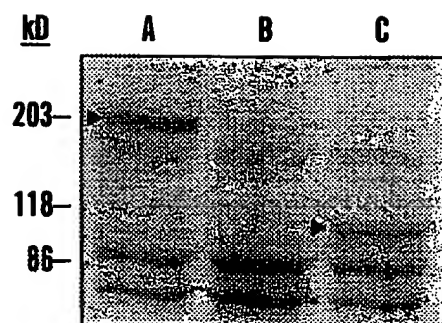


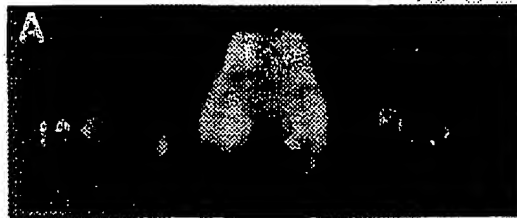
FIG. 1D

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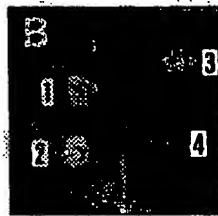


**FIG. 2**

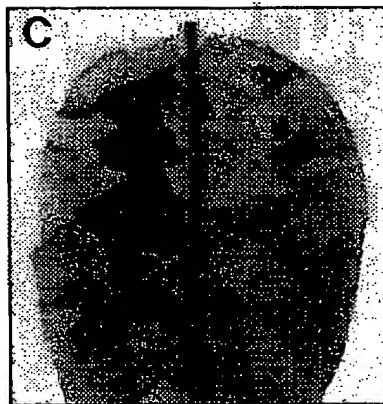
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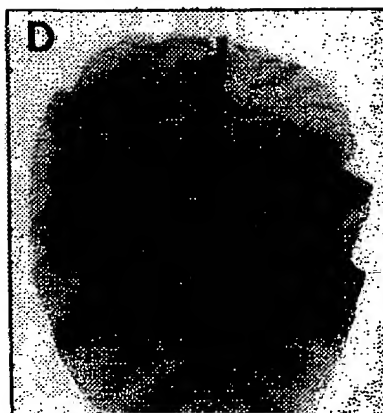
**FIG. 3A**



**FIG. 3B**

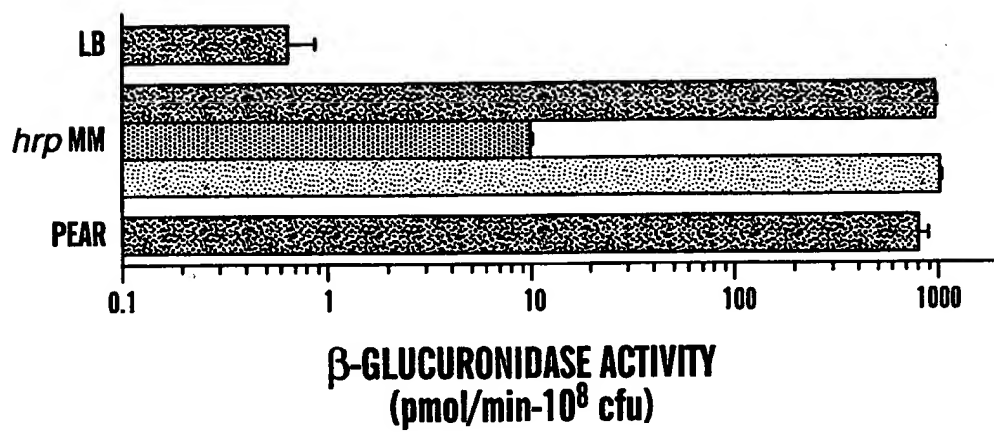


**FIG. 3C**

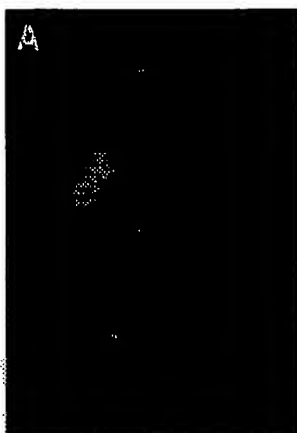


**FIG. 3D**

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**FIG. 4**

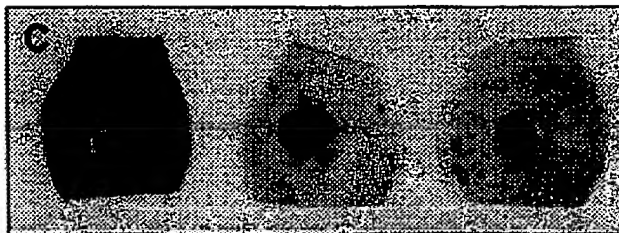
5/5



**FIG. 5A**



**FIG. 5B**



**FIG. 5C**

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US98/15426

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) : A01G 13/00; A61K 35/66; C12N 1/20; C12R 1/18

US CL : 530/350; 536/23.7; 435/874; 800/301

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/350; 536/23.7; 435/874; 800/301

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

BIOSIS, MEDLINE, AGRICOLA, CAPLUS

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WEI et al. Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen Erwinia Amylovora. Science. 1992, Vol. 257, pages 85-88, see entire document.	1-16, 29-37
Y	BURR et al. Increased Potato Yields by Treatment of Seedpiece with Specific Strains of Pseudomonas Fluorescens and P. Putida. Phytopathology. 1978, Vol. 68, pages 1377-1383, see entire document.	1-16, 29-37

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*B* earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z* document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means	
*P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

24 OCTOBER 1998

Date of mailing of the international search report

03 DEC 1998

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**INTERNATIONAL SEARCH REPORT**

International application No.

PCT/US98/15426

**C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<b>BAILLIEUL et al. A New Elicitor of the Hypersensitive Response in Tobacco: A Fungal Glycoprotein Elicits Cell Death, Expression of Defence Genes, Production of Salicylic Acid, and Induction of Systemic Acquired Resistance. The Plant Journal. 1995, Vol. 8, No. 4, pages 551-560, see entire document.</b>	<b>1-16,29-37</b>

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US98/15426

## Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:  
1-16,29-37

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US98/15426

### BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

**Group I.** Claims 1-16, 29-37 are drawn to a DNA molecule s encoding the hypersensitive eliciting protein, transformation and resulting transgenic plants.

**Group II:** Claims 17-28, 38-39 are drawn to an isolated hypersensitive eliciting response eliciting protein.

**Group III.** Claims 40-43 are drawn to antibody.

The inventions listed as groups I-III do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The specific technical feature of group 1 is a transgenic plant with improved disease resistance as a result of the expression of transgenes encoded by SEQ ID NO:1 and 3. Second product does not require the special technical features of group 1 because it entails the use of protein and it does not require the particular DNA molecules of group 1. The third product of group III is antibody, not required by group 1. Since group 1 involves the use of expression of transgene in transgenic plants and as such do not share the same technical features. Thus, the claims are not so linked by a special technical feature within the meaning of the PCT Rule 13.2 so as to form a single inventive concept, accordingly, the unity of invention is lacking among all groups.